Wandering albatross, *Diomedea exulans*, and the flightless moth, *Pringleophaga marioni*, on sub-Antarctic Marion Island: A case of thermal ecosystem engineering?

by

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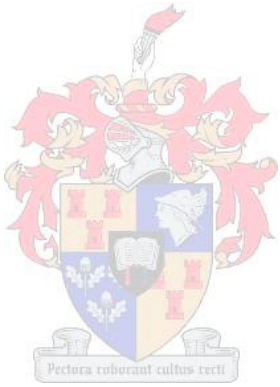

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Abstract

Recent work has shown that on sub-Antarctic Marion Island, caterpillars of the flightless tineid moth, *Pringleophaga marioni*, have much higher and considerably less variable populations in recently abandoned nests of the wandering albatross, *Diomedea exulans*, compared to old nests and other plant communities. Since no evidence for nutrient input was provided, it was hypothesised that wandering albatrosses serve as thermal ecosystem engineers by providing a warm microhabitat in which caterpillar growth and survival are improved. In this thesis, I used a multidisciplinary approach integrating physiology, ecology and behaviour, to better understand the reason for the high caterpillar biomass in nests, and explore the hypothesis of thermal ecosystem engineering.

My first objective was to provide a more quantitative life-cycle estimate for *P. marioni* by rearing caterpillars at different temperature regimes, and in so doing estimate the effects of temperature on development and survival (Chapter 2). Contrary to previous estimates of 2-5 years, a year-long life cycle estimate was found, and although development was fastest at high temperatures of 15°C, caterpillars had low survival. Development time was similar at the fluctuating temperatures of 5-15°C and 10°C, with a longer duration at 5°C.

By conducting a more extensive sampling effort of caterpillar biomass and temperature in nests (Chapter 3), I showed that recently abandoned nests had a significantly higher abundance of caterpillars compared to nests from which chicks had recently fledged, as well as older nests. Temperature data collected over a *c.* one year period showed that temperature in occupied nests remained high during the entire year of occupancy and events at which *P. marioni* experience chill coma were substantially reduced.

Consequently, the effects of thermal acclimation on the physiological and behavioural responses of *P. marioni* caterpillars were explored. First, how temperature affects the metabolic rate of caterpillars was examined (Chapter 4). Metabolic rate was significantly higher at a low acclimation temperature of 5°C compared to 15°C, providing partial support for metabolic cold adaptation. No evidence was provided that caterpillars reduce their metabolic rates to conserve water, and caterpillars responded to fluctuating temperatures by depressing their metabolic rates. Second, the relationship between thermal preference and locomotor performance of caterpillars was examined (Chapter 5). A common assumption made is that animals will prefer temperatures that maximise performance. Preference was significantly lower (*c.* 8°C) compared to the optimum temperature for locomotion (*c.* 23°C), and it was suggested that caterpillars may prefer lower temperatures where survival or

assimilation efficiency is maximised. Lastly, if nests provide a fitness advantage, either caterpillars or ovipositing female moths may likely seek out nests. Using choice experiments (Chapter 6), I showed that caterpillars are unlikely to use thermal or chemosensory cues to locate nests and showed a strong avoidance to high temperatures of 15°C. This latter response initially does not appear to fit with the idea that caterpillars favour warm nest temperatures.

Although such high temperatures may occasionally be reached in wandering albatross nests, there is considerable daily fluctuation. Variable temperatures including high temperatures of 15°C did not have deleterious effects on caterpillar growth rates and survival, and development was fastest at the fluctuating temperature of 5-15°C compared to 5°C. Caterpillars may also experience chill coma events less often in warm nest environments. Collectively, these findings suggest that the thermal engineering by wandering albatross may indeed improve caterpillar growth and survival. Additional factors were also identified that may contribute to the high abundance of caterpillars in nests (e.g. differential mortality in and out of nests because of mouse predation; high temperatures enhance decomposition rates) (Chapter 7), therefore leaving much scope for future work to further explore this unique interaction between wandering albatross and flightless moth caterpillars.

Opsomming

Onlangse werk het getoon dat op sub-Antarktiese Marion Eiland ruspes van die vlerklose mot *Pringleophaga marioni* baie hoër en aansienlik minder veranderlike bevolkings het in onlangs verlate neste van die Grootalbatros, *Diomedea exulans*, in vergelyking met die ou neste. Aangesien daar geen bewyse vir voedingstof insette is nie, is die hipotese dat die Grootalbatros dien as ‘n termiese ekosisteem ingeneur deur die verskaffing van 'n warm mikrohabitat waarin ruspe oorlewing en groei verbeter. In hierdie tesis gebruik ek 'n multi-dissiplinêre benadering deur fisiologie, ekologiese en gedrag te integreer om die rede vir die hoë ruspe biomassa in neste beter te verstaan, en die hipotese van 'n warm ekosisteem ingeneur te verken.

My eerste doelwit was om 'n meer kwantitatiewe lewensiklus skatting vir *P. marioni* te voorsien deur ruspes by verskillende temperatuur groot te maak, en sodoende die effek van temperatuur op die ontwikkeling en oorlewing te bepaal (Hoofstuk 2). In teenstelling met die vorige skattings van 2-5 jaar, is daar tans ‘n jaar lank lewensiklus skatting gevind, en hoewel ontwikkeling die vinnigste was by hoë temperature van 15°C, het die ruspes lae oorlewing vertoon. Ontwikkelingstyd was soortgelyk by wisselende temperature van 5-15°C en 10°C, met 'n langer duur by 5°C.

Deur die uitvoer van 'n meer intensiewe monsterneming van ruspe biomassa en temperatuur in neste (Hoofstuk 3), het ek getoon dat onlangs verlate neste 'n aansienlike hoër aantal ruspes het in vergelyking met die neste van waar kuikens onlangs volwaardig was, sowel as ouer neste. Temperatuur data versamel oor 'n tydperk van ongeveer een jaar het getoon dat die besette neste se temperatuur hoog gebly het gedurende die hele jaar van besetting.

Gevolglik is die effek van 'n warm akklimasie op die fisiologiese en gedrags reaksies van *P. marioni* ruspes verken. Eerstens is die effek van hoë temperatuur op die metaboliese tempo van ruspes ondersoek (Hoofstuk 4). Metaboliese tempo is aansienlik hoër na 'n lae akklimasie temperatuur van 5°C in vergelyking met 15°C, die verskaffing van gedeeltelike steun vir metaboliese koue aanpassing. Geen bewyse is gevind dat die ruspes hul metaboliese tempo verminder om water te bewaar nie, en ruspes reageer op wisselende temperature deur hul metaboliese tempo te verlaag. Tweedens is die verhouding tussen hitte voorkeur en die bewegings prestasie van ruspes ondersoek (Hoofstuk 5). ‘n Algemene aanname is dat diere temperature sal verkies waar hulle maksimum sal presteer. Voorkeur was aansienlik laer (*c.*

8°C) in vergelyking met die optimum temperatuur vir beweging (*c.* 23°C), en dit is voorgestel dat ruspes laer temperature waar oorlewing of assimilasie doeltreffendheid geoptimaliseer word kan verkies. Ten slotte, indien neste 'n fiksheid voordeel inhou, sal ruspes of eierleênde vroulike motte waarskynlik neste uitsoek. Deur die gebruik van keuse eksperimente (Hoofstuk 6), het ek getoon dat ruspes onwaarskynlik hitte of chemosensoriese leidrade gebruik om neste op te spoor en het 'n sterk vermyding aan hoë temperature van 15°C. Die laasgenoemde reaksie is in kontras met die idee dat ruspes ten gunste is van warm nes temperature.

Alhoewel sulke hoë temperature soms bereik kan word in Grootalbatros neste, is daar ' n aansienlike daaglikse vernadering. Veranderlike temperature insluitende hoë temperature van 15°C het nie nadelige uitwerking op die ruspe se groei en ontwikkeling nie en is die vinnigste by die wisselende temperature van 5-15°C in vergelyking met 5°C. Die hoë optimale veranderlike temperatuur dui daarop dat ruspes meer aktief kan wees in warm neste wat lei tot verhoogde verbruik en groei. Ruspes kan ook minder dikwels verkillingskoma ervaar in warm nes omgewings. Gesamentlik dui hierdie bevindinge daarop dat die hitte ekosisteem ingeneur deur die Grootalbatros inderdaad ruspe oorlewing en groei kan verbeter. Daar is egter ander faktore wat geïdentifiseer is wat ook kan bydra tot die hoë oorvloed van ruspes in neste (bv. differensiële mortaliteit in en uit die neste as gevolg van muis predasie, hoë temperature verhoog ontbindingstempo) (Hoofstuk 7), dus laat ruimte vir toekomstige werk.

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Chapter 1 – General Introduction

"Wherever a large bird colony exists, the birds may be regarded as dominants in the sense that…they determine the nature of the community of the area occupied by the colony."
(Hutchinson 1950, p. 369)

Wandering albatross, *Diomedea exulans*, offshore Marion Island
Photo credit: L. Peck

Marine-terrestrial links

Ecosystems do not exist independently of each other, but are open to the flow of nutrients, energy, and materials between them. Thus, despite the boundaries that exist between land and ocean, marine and terrestrial habitats interact extensively and significantly to form one of the world's major ecosystems. Oceanographers have long studied how "terrigenous input" (i.e. material derived from land) affects marine primary and secondary productivity. Nutrients, trace elements, organic material and living prey enter the ocean directly from land and indirectly via rivers and estuaries (Barnes & Hughes 1988; Balls 1994; Croot & Hunter 1998; Jickells *et al.* 2000; Neal & Davies 2003). In addition, aerosol transfers from the land are major pathways of nutrient delivery to the oceans (Duce & Tindale 1991; Fung *et al*. 2000; Xia & Gao 2010). These terrestrial inputs may result in high primary and secondary marine productivity (Martin *et al.* 1989; Lohrenz *et al.* 1999; Dagg & Breed 2003; McKee *et al*. 2004; Schlacher *et al.* 2009).

Likewise, energy and nutrients also flow from productive marine systems to generally unproductive terrestrial systems (Polis *et al.* 1997; Anderson & Polis 1999). Marine aerosols that contain rare micronutrients and organic compounds travel inland onto continents and this may have positive effects on local soils and plant productivity (Polis *et al.* 2004; Power *et al.* 2004). Similarly, shore drift of algae and carrion, marine mammals (e.g. seal colonies) and, of particular interest to this study, sea birds; are all major contributors to energy transfer between marine and terrestrial systems (Smith 1978; Lindeboom 1984; Hawes 1990; Polis & Hurd 1996; Bokhorst *et al.* 2007). By coming ashore to breed or to moult, the primary role of seabirds in these ecological interactions is the transfer of marine derived nutrients and energy. The significance of seabirds in linking marine and terrestrial systems is therefore widely recognized (Heatwole 1971; Mizutani & Wada 1988; Polis & Hurd 1996; reviews in Polis *et al.* 1997 and Ellis 2005), particularly in the nutrient-poor high Arctic regions (Keatley *et al.* 2009; Michelutti *et al.* 2009; Choy *et al.* 2010; Griffiths *et al.* 2010; Zmudczyńska *et al.* 2012; Zmudczyńska-Skarbek 2013) and islands across the globe (e.g. Polis & Hurd 1995; 1996; Anderson & Polis 1998; 1999; Stapp *et al.* 1999; Sánchez-Piñero & Polis 2000; Kolb *et al.* 2012).

Seabird colonies affect terrestrial communities in four general ways: directly, via (1) nutrients derived from guano that enhance plant primary productivity (e.g. Heatwole 1971; Onuf *et al.* 1977; Ryan & Watkins 1989; Anderson & Polis 1999; Sánchez-Piñero & Polis 2000; Wait *et al.* 2005); (2) seabird carrion such as eggs or the bodies of chicks and adults which serve as a food source for many coastal scavengers (e.g. Siegfried *et al.* 1978;

Williams *et al.* 1978; Polis & Hurd 1995; Sánchez-Piñero & Polis 2000); (3) living seabird tissue on which many parasites and predators feed (e.g. Duffy 1983; 1991; Polis & Hurd 1995); and (4) indirectly via the food web when populations of secondary and primary consumers benefit from the enhanced effects of primary productivity (e.g. Onuf *et al.* 1977; Polis & Hurd 1995; Stapp *et al.* 1999; Barret *et al.* 2005). Seabird colonies therefore often support plant and invertebrate communities that are more abundant and species-rich compared to those uninfluenced by seabirds (e.g. Onuf *et al*. 1977; Sánchez-Piñero & Polis 2000; Barrett *et al.* 2005; Maron *et al.* 2006; Kolb *et al.* 2012; Zmudczyńska *et al.* 2012). These effects are particularly prominent on small islands which receive more marine energy, are more productive per unit area, and generally have fewer predators of nesting birds (Heatwole 1971; Polis & Hurd 1995; 1996).

Of all seabird influences, the effects of guano are by far the most widely recognized (Hutchinson 1950; Burger *et al.* 1978; Bosman & Hockey 1988; Wootton 1991; Wainright *et al.* 1998; Wait *et al.* 2005). The effects of guano are apparent in many different ecosystems, such as polar regions, desert islands and freshwater ponds (Harding *et al.* 2004; Wait *et al.* 2005; Keatley *et al.* 2009). Seabirds feed on fish and marine invertebrates that are rich in nutrients. The high concentrations of phosphate, nitrate and ammonia (Hutchinson 1950; Wainright *et al.* 1998; Anderson & Polis 1999) found in guano therefore serve as a rich fertilizer that not only enhances the productivity of land plants, but also changes plant species composition both positively (e.g. Ellis *et al.* 2006) and negatively when high nutrient concentrations become toxic (e.g. Wait *et al.* 2005; Kolb *et al.* 2012). Increases in algal production have also been associated with high nutrient concentrations derived from seabird guano, and in consequence, intertidal community structures are modified (Bosman *et al.* 1986; Bosman & Hockey 1988). These studies have highlighted the importance of seabirds in structuring and supporting terrestrial communities, and this is further realised by the substantial loss or decline of plant or animal communities on islands where seabird numbers have been depleted (e.g. Norton *et al.* 1997; Fukami *et al.* 2006; Mulder *et al.* 2009; Towns *et al.* 2009).

Ecosystem engineering

Seabirds influence terrestrial systems in many ways, but particularly as ecosystem engineers (overview of ecosystem engineering in Cuddington *et al.* 2007). The latter are organisms that directly or indirectly modulate the availability of resources to other species through their actions or presence by changing biotic or abiotic materials, and in so doing, create, modify or

maintain habitats (Jones *et al.* 1994; 1997; Wright & Jones 2006; Hastings *et al.* 2007). The iconic example of ecosystem engineering is beaver dams. Beavers build dams which create ponds that have profound effects on resource flows (water, sediments and nutrients) creating habitats for a diverse range of organisms (Naiman *et al.* 1986; Naiman *et al.* 1994; Wright *et al.* 2002). In their review of ecosystem engineering, Jones *et al.* (1994) used beaver dams as an example of a habitat or resource being modified, and mussel beds modifying wave action and therefore erosion, as an example of abiotic changes that alter resource use or availability.

A critical characteristic of ecosystem engineering is that it changes the availability (i.e. quality, quantity and distribution) of resources utilized by other taxa (Jones & Gutiérrez 2007). The direct provision of resources by an organism to other species in the form of living or dead tissue is not engineering (Jones *et al.* 1997). Under this scheme, nutrient input via seabird colonies is not ecosystem engineering, since the birds are directly providing a resource. Instead, seabirds contribute to ecosystem engineering largely through the excavation of burrows and the construction of other forms of nests. The favourable microhabitats of seabird nests or burrows and the services of the builders may create habitat niches which attract a variety of organisms (Joly *et al.* 1987; Newman 1987; Bancroft *et al.* 2004; 2005; McKechnie 2006; Moravcová *et al.* 2010; but see Mulder & Keall 2001 and Vincke *et al.* 2007).

Seabirds on the Southern Ocean Islands

Nutrient input

The importance of seabirds in nutrient transfer on the Southern Ocean Islands (SOI) has long been documented (Smith 1977; 1978; 1979; Burger *et al.* 1978; Williams & Berruti 1978; Williams *et al.* 1978; Siegfried 1981; Erskine *et al.* 1998; Vidal *et al.* 2003). Many of these studies formed part of the International Biological Program (IBP) of which the work on sub-Antarctic Marion Island (part of the Prince Edward Island group, see below) was recently summarized by Smith (2008). The sub-Antarctic region is generally defined by the presence of closed phanerogamic communities, but completely lacking trees or woody shrubs (Wace 1965).

Seabird contributions to nutrient input on Marion Island are in the form of eggs (Siegfried *et al*. 1978), feathers (Williams & Berruti 1978), carcasses (Williams *et al*. 1978) and guano (Burger *et al*. 1978, Smith 1978; 1979; Lindeboom 1984). Collectively, these studies have shown that seabirds are key contributors to the island's terrestrial habitats by providing mineral and energy sources (reviews in Siegfried 1981; Smith 2008). Guano, in

particular, represents an annual input of 640 tonnes N, 106 tonnes P, 84 tonnes K, 222 tonnes Ca, and 21 tonnes Mg to the island's terrestrial ecosystem (Smith 2008). Much of these nutrients are presumably leached back into the sea (Burger *et al.* 1978), however the effects of guano can be striking and are evident in the vibrant colours and luxurious growth of vegetation in the immediate vicinity of penguin colonies and around the nests of other seabirds (see Fig. 1) (Smith 1977). From penguins to small burrowing petrels, larger albatrosses and giant petrels, all of the surface and sub-surface nesters make significant contributions of nutrient transfers, with further influences on the invertebrate biota and ecosystem functioning on Marion Island (Smith 1976; 1977; 1978; 1979; Burger *et al.* 1978; Crafford & Scholtz 1986; Gabriel *et al.* 2001; Barendse *et al*. 2002).

Fig. 1. A wandering albatross (*Diomedea exulans*) nest surrounded by *Poa cookii* tussocks. Photo credit: A. Phiri

Wandering albatross as ecosystem engineers

On Marion Island, caterpillars of the flightless moth, *Pringleophaga marioni* Viette (Lepidoptera: Tineidae) (Viette 1949), are keystone species in nutrient cycling (Smith & Steenkamp 1992; 1993) and recent work has shown that caterpillars have much higher and considerably less variable populations in recently abandoned wandering albatross, *Diomedea exulans*, nests than in old nests and in most other plant communities (Sinclair & Chown 2006). Joly *et al.* (1987) suggested that the large numbers of invertebrates that occur in wandering albatross nests on Ilê de la Possession are as a result of nutrient input. Indeed, on Marion Island, wandering albatross deposit 2.88 kg (dry mass) of guano at each nest annually

(Burger *et al.* 1978) and soils surrounding occupied and abandoned nests have high concentrations of N (Smith 1978). These levels vary depending on the presence or absence of the birds at the nest. Concentrations of N are high following nest establishment and even higher once eggs are laid. Upon hatching, a five-fold increase can be found. At this stage, soils also often possess high concentrations of Ca and Cl^- (Croome 1972, unpublished expedition report).

However, a recent study by Sinclair & Chown (2006) showed that N and other nutrients did not differ significantly between wandering albatross nests and other habitats on the island. There was also no significant difference in nutrient concentrations between old nests and nests that had been recently abandoned (Sinclair & Chown 2006). Nests are elevated high above the surrounding substrate and are subjected to greater nutrient loss via leaching (Smith & Steenkamp 1992). These findings suggest that nutrient input by wandering albatross do not contribute to the significantly higher biomass of *P. marioni* caterpillars found in nests. Instead, Sinclair & Chown (2006) suggested another reason for this occurrence, that of ecosystem engineering. Incubating albatross adults and chicks raise nest temperatures such that occupied nests are 5°C warmer compared to abandoned nests and soils (Sinclair & Chown 2006). The optimum temperature for *P. marioni* feeding is at 10°C (Crafford 1990), and since repeated exposure to sub-zero temperatures results in cessation of feeding (Sinclair & Chown 2005), elevated temperatures may allow for continued growth, particularly during winter. In turn, caterpillar growth, as well as survival in warmer nest environments may be improved (Sinclair & Chown 2006). In addition, when winter soil temperatures drop to below zero, warmer nest temperatures may keep microhabitat temperatures well above the chill coma temperature of -0.6°C for this species (Klok & Chown 1997). By keeping caterpillars warm, Sinclair & Chown (2006) suggested that wandering albatross may serve as thermal ecosystem engineers which may account for the high caterpillar biomass in nests.

Temperature effects on ectotherms

For *Pringleophaga marioni* and other ectotherms, temperature is indeed one of the most important factors influencing biological processes (Chown & Nicolson 2004; Chown & Terblanche 2007; Angilletta 2009). Environmental temperature is therefore key in virtually all aspects of ectotherm behaviour and physiology (Angilletta *et al.* 2002; Chown & Nicolson 2004). This includes growth (Savage *et al.* 2004), locomotion (Huey & Stevenson 1979; Angilletta *et al.* 2002) and metabolic rate (Brown *et al.* 2004). The response of ectotherms to temperature variation in any environment is therefore crucial for survival, and this is often

accomplished via phenotypic alterations (e.g. morphological or physiological) (Fordyce 2006). When this process of change in response to environmental variation involves physiological traits, it is often referred to as acclimation (in the laboratory) or acclimatization (in the field) (Fordyce 2006; Kingsolver & Huey 1998). Because the variability and predictability of environmental temperature can be readily quantified (Kingsolver & Huey 1998), studies of thermal acclimation have long been a topic of physiological research (Prosser 1955), and have since become an important means by which the response of organisms to environmental variation has been explored (Huey *et al.* 1999; Wilson & Franklin 2002; Woods & Harrison 2002; Slabber *et al*. 2007).

Performance curves are often used to describe the relationship between physiological performance and temperature. These are characterized by quantifying upper and lower critical temperatures, the thermal performance breadth (range of temperatures which permit performance), and the optimal temperature (temperature at which performance is maximised) (Huey & Stevenson 1979). The impact of acclimation of a thermal performance curve will be manifest as a change in the position or shape of the curve, as evidenced for example by a shift in the optimal temperature (Angilletta 2006). An assumption often made is that organisms will prefer body temperatures at which performance is optimal (Angilletta *et al.* 2006). Termed coadaptation, physiological ecologists have long assumed that thermoregulatory behaviour and thermal physiology are closely matched (reviews in Angilletta *et al.* 2002; Angilletta *et al.* 2006; Angilletta 2009). However, departure from coadaptation has also been shown, with findings of preferred temperature being lower than optimum temperature in a range of ectotherms including reptiles and insects (Martin & Huey 2008).

The effects of temperature on sub-Antarctic ectotherms

Sub-Antarctic islands are persistently cool year-round (*c.* 6.5°C) (Le Roux & McGeoch 2008), and seasonal variation is relatively low because of oceanic influences (Convey 1996a). This allows for activity and development to occur continuously throughout the year (Convey (1996a), suggesting that organisms have developed a suite of behavioural and physiological strategies which allow them to cope in these conditions. One such strategy is that of metabolic cold adaption (Clarke 1993). Ectotherms may compensate for low temperature conditions by elevating their metabolism (Clarke 1993; Chown & Gaston 1999; Addo-Bediako *et al.* 2002; Clarke 2003), a strategy which may allow for growth, development and completion of life-cycles despite low temperature conditions (Sømme & Block 1991). Indeed, some organisms on Marion Island and other sub-Antarctic islands have shown some

form of respiratory adaptation to cold conditions (e.g. Sømme *et al.* 1989; Crafford & Chown 1993; Chown *et al.* 1997).

Whereas metabolic cold adaption predicts an elevated metabolic rate in cold conditions, the opposite is predicted in warmer conditions – i.e. ectotherms, and insects in particular, may depress their metabolism such that respiratory water loss is minimised (Addo-Bediako *et al.* 2001; Chown 2002). With the generally damp cool climates of the sub-Antarctic, water conservation may not be of great ecological significance (Convey 1996a). However, despite this, both plants (*Arctotheca populifolia*) (Ripley *et al.* 1999) and invertebrates on Marion Island: weevil larvae, *Bothrometopus randi* (Chown & Van Drimmelen 1992) and *Embryonopsis halticella* caterpillars (Klok & Chown 1998), have shown a considerable resistance to desiccation mainly as a result of very high wind speeds and limited boundary layers (Klok & Chown 1998). Climate change is also occurring rapidly in these regions, and on Marion Island, has already resulted in an increase in temperature and decrease in precipitation (Smith & Steenkamp 1990; Smith 2002; Mélice *et al.* 2003; Le Roux & McGeoch 2008). Thus, if habitats continue to dry, water stress may likely prove a significant factor influencing life-forms in the sub-Antarctic, and selection for water conservation may become particularly important (Klok & Chown 1997; Chown *et al.* 2011).

Furthermore, despite the relatively stable climates of the sub-Antarctic, environmental unpredictability is common (Deere *et al.* 2006), meaning that environmental cues may lead to inappropriate responses (Chown & Convey 2007; Chown & Terblanche 2007). Acclimatization in these unpredictable environments may not be selectively advantageous as environmental cues are likely to be poor predictors of future environmental conditions (Deer & Chown 2006). This may be the primary reason why some invertebrates on Marion Island have shown no or very little response to acclimation (e.g. Klok & Chown 1998; Deer & Chown 2006; Deere *et al.* 2006; Slabber *et al.* 2007; Marais *et al.* 2009). Nonetheless, some acclimation responses to physiological traits have been observed (e.g. Klok & Chown 1997; Slabber & Chown 2004; Chown *et al.* 2007; Jumbam *et al.* 2008). Together, these studies suggest that laboratory tests provide an efficient and effective way to investigate thermal acclimation and have become a primary means by which the consequences of temperature variation on ectotherm performance can be explored on Marion Island and other sub-Antarctic Islands.

Study site and species description

Marion Island

Marion Island (46°54'S, 37°45'E) forms part of the Prince Edward Island group and is located in the South Indian Ocean approximately 2300 km southeast of Cape Town, South Africa (Fig. 2). Neighbouring Prince Edward Island (46°38'S, 37°57'E) is the smaller of the two islands (i.e. 45 km^2 compared to 270 km^2 for Marion Island) with a stretch of 19 km between the two islands (Chown & Froneman 2008). Of volcanic origin, the islands are fairly young, approximately 500 000 years old (McDougall *et al.* 2001). The islands have an oceanic climate with persistently low temperatures, high humidity, and strong winds. Mean annual air temperatures are a low *c*. 6.5°C, and annual precipitation is high at *c*. 1900 mm. Temperatures are relatively stable ranging from 2°C in summer to 7°C in winter. Subzero temperatures occur throughout the year and with high cloud cover, only 25-30% sunshine occurs annually. Because of global warming, the climate has changed substantially, with a mean annual temperature increase of more than 1°C and a decline in precipitation of more than 500 mm per annum (Smith & Steenkamp 1990; Smith 2002; Mélice *et al.* 2003; Le Roux & McGeoch 2008).

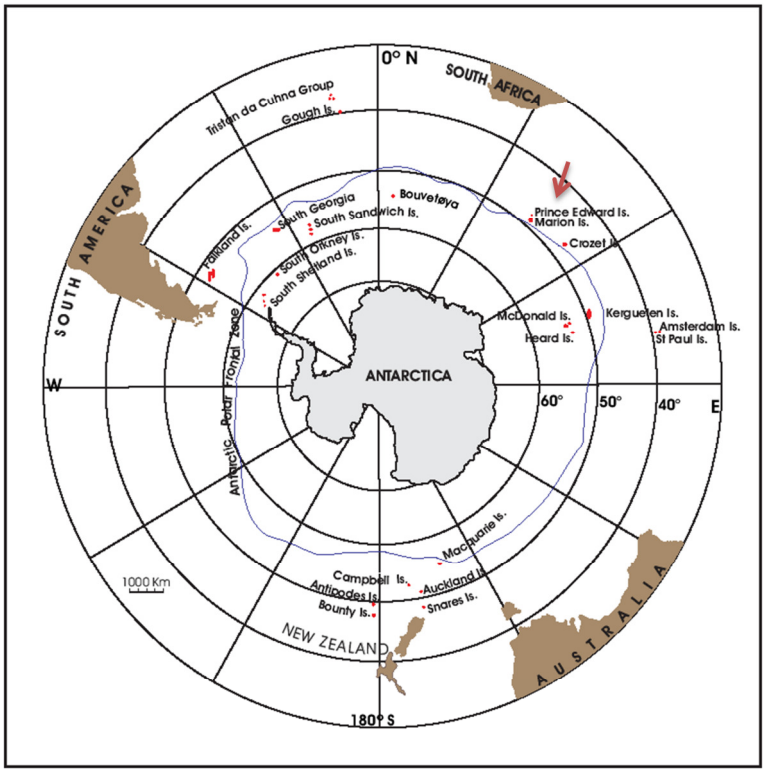

Fig. 2. Map showing the position of the Prince Edward Island group (indicated by the red arrow) and other Southern Ocean Islands (adapted from Chown *et al.* 2008).

There are two major biomes on Marion Island: tundra which predominates in lowland areas, and barren polar desert at higher elevations. The vegetation has been divided into six habitat complexes, namely mire, slope, fellfield, biotic, saltspray and polar desert (Gremmen & Smith 2008). Because of the large distance from any other continent, the island is species-poor. There are no indigenous land mammals, Lesser Sheathbills, *Chionis minor*, are the only land birds, and apart from *P. marioni*, 63 free-living and 39 introduced invertebrate species have been recorded on the island (Chown & Froneman 2008). Marine visitors to the island include 27 seabird species and marine mammals such as elephant seals (*Mirounga leonina*), fur seals (*Arctocephalus tropicalis*) and Antarctic fur seals (*Arctocephalus gazella*) that visit the island to breed and moult. Of the 35 vascular plant species, 12 were introduced, while bryophytes are slightly more species-rich with 134 species (see Chown & Froneman 2008 for overview).

Terrestrial herbivory is uncommon and invertebrates are mostly responsible for nutrient turnover (Holdgate 1977; Burger 1985; Convey 1996b; Hogg *et al.* 2006). The large densities of soil macroinvertebrates such as earthworms, moth and weevil larvae, speed up nutrient cycling by feeding on the vast quantities of slowly decomposing organic matter in the soils and peats of the island (Smith 1977; Smith & Steenkamp 1992; 1993). Peat contains the highest nutrient reserve in the island's ecosystem (Smith & Steenkamp 1993), but N, P, K and Ca accumulate in peat deposits and are inaccessible for primary production (Smith 1977). Soil macroinvertebrates therefore speed up nutrient cycling by fragmenting litter, thereby increasing the surface area for microbial activity (Smith & Steenkamp 1992). Moth and weevil larvae are the dominant detritivores in the Islands vegetated zones (Crafford 1990), with *P. marioni* being a key contributor to nutrient cycling (Fig. 3) (Smith & Steenkamp 1992; 1993).

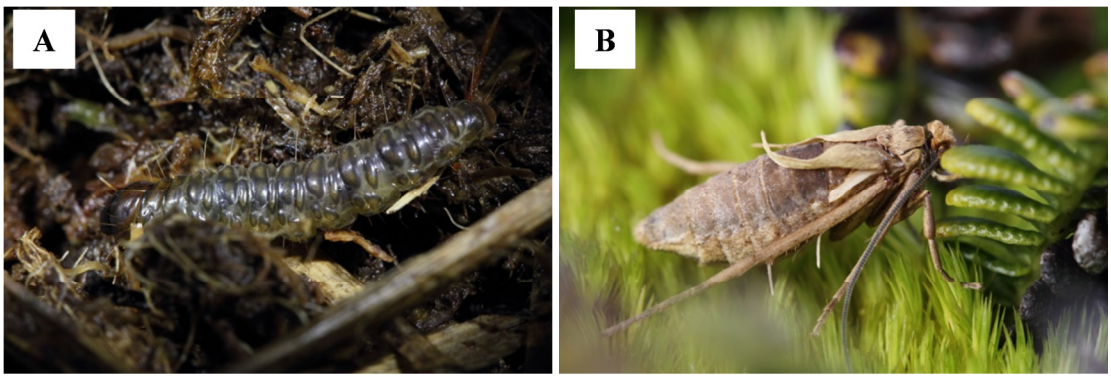

Fig. 3. Larvae (A) and adult female (B) of *Pringleophaga marioni*.
Photo credit: O. Whitehead.

Pringleophaga marioni larvae greatly stimulate the release of nutrients from peat and litter and are responsible for substantial increases in nutrient mineralization, processing in excess of 100 kg.ha^{-1} dry mass of litter annually (Smith & Steenkamp 1992; 1993). Caterpillars have rich and varied intestinal bacteria which may account for these high decomposition rates (Crafford 1987; Crafford 1990). Although *P. marioni* larvae are detritivorous, individuals held in the laboratory have also shown some degree of omnivory, including both earthworms and conspecifics in their otherwise typical litter diet (French & Smith 1983; Crafford 1990). A threat to populations of *P. marioni*, and thus indirectly, to ecosystem functioning, is the introduced house mouse, *Mus musculus* (Smith & Steenkamp 1990; Chown & Smith 1993; Bergstrom & Chown 1999; Smith 2002). The mice take *Pringleophaga* caterpillars as their preferred food source (Rowe-Rowe *et al.* 1989; Smith *et al.* 2002; but see Chown & Smith 1993), and are responsible for population declines of *P. marioni* on Marion Island (Smith & Steenkamp 1990; Chown & Smith 1993; Bergstrom & Chown 1999; Smith 2002, but see Van Aarde *et al.* 2004). Nonetheless, *P. marioni* can still be found in a range of habitats on Marion Island, from saltspray plant communities on the coast, microhabitats under stones, altitudes up to 800 m, and of particular importance to this study, in the nests of wandering albatross (Crafford *et al.* 1986; Sinclair & Chown 2006).

Wandering albatrosses are the largest flying seabirds nesting on SOI (Warham 1997) where they are IUCN red listed species (Cooper *et al.* 2006). Breeding in loose colonies, their nests occur scattered around the islands with average densities of 22 nests ha^{-1} on Prince Edward Island and 5 nests ha^{-1} on Marion Island (Underhill *et al*. 2003). Nests (*c*. 0.1-0.25 m^3) are large mounds of vegetation and peat collected from the surrounding area. Nests are ephemeral (lasting a year) – a single egg is laid in mid-summer, hatches in March, and chicks fledge from December to February (Ryan & Bester 2008). Wandering albatross maintain a body temperature of 38°C (Warham 1997). That nests serve as warm microhabitats for organisms adversely affected by sub-zero temperatures (Sinclair & Chown 2005) is therefore easily envisaged.

Thesis outline

In this thesis, I adopt a multidisciplinary approach in which the physiology, ecology and behaviour of *P. marioni* caterpillars are examined, with the primary goal being to further explore the role of wandering albatross as thermal ecosystem engineers, and to gain a better understanding as to why caterpillars occur abundantly in albatross nests. Possible ultimate

explanations include nutrient (see Joly *et al.* 1987) and thermal (Sinclair & Chown 2006) benefits once caterpillars are in nests. Proximate explanations are the mechanisms by which caterpillars arrive in nests, which do not preclude either nutrient or thermal benefits once caterpillars are in the nests. These are: nest-finding by caterpillars or ovipositing female moths, incidental incorporation into nests, either during nest-building, since albatross pairs build their nests from the surrounding vegetation (Ryan & Bester 2008), or, ovipositing female moths may distribute their eggs everywhere, but survival is improved in nests. Another explanation is differential mortality – i.e. moths may distribute their eggs everywhere, but caterpillars may be predated upon by mice less often in nests compared to other habitats.

Since it has already been found that nutrient availability plays an insignificant role in explaining the high caterpillar abundance in nests (Sinclair & Chown 2006), I focussed my attention on thermal ecosystem engineering by setting out the following hypotheses: (i) caterpillar abundance in nests is significantly higher and less variable compared to surrounding habitats; (ii) elevated temperatures typical of occupied nests result in improved caterpillar performance, and hence, survival; (iii) because caterpillar fitness is elevated in nests, either ovipositing female moths or caterpillars may preferentially seek out nests.

If these hypotheses are met, insufficient evidence would be provided to reject the thermal ecosystem engineering hypothesis. Each chapter is a stand-alone study written and formatted with its own introduction, methods, results and discussion, whilst chapter seven provides a synthesis of the previous chapters.

- Long life cycle estimates of two to more than five years have been proposed for *P. marioni* (Crafford *et al.* 1986; Crafford 1990). An ongoing puzzle is why the species has not shown greater population declines on Marion Island than have been recorded given extremely high estimates of mouse predation. One reason may be inaccurate life cycle estimates. In **Chapter 2**, this puzzle was finally solved by rearing caterpillars in different temperature regimes to provide a new quantitative life-cycle estimate for *P. marioni*, and in so doing, the effects of temperature on development and survival were examined.
- Previous work by Sinclair & Chown (2006) examined caterpillar biomass in two nest categories: recently abandoned (new) and old. However, nests that were occupied the season prior to sampling and from which chicks had fledged were not sampled. In the

prior study, temperature data were collected over a period of two weeks, whereas nests are occupied for a full year. In **Chapter 3**, I conducted a more extensive sampling effort of caterpillar biomass in all three nest types (old, new and fledged) to determine the effects of nest age. In addition, temperature in occupied nests, abandoned nests and surrounding soils were recorded over a full year.

- Ectotherms such as insects have developed various strategies to cope with environmental variation. By acclimating caterpillars to varying temperature regimes and then examining their metabolic rate and water loss rate response, the theories of metabolic cold adaptation (Chown & Gaston 1999; Addo-Bediako *et al.* 2002; Clarke 2003) and water conservation (Zachariassen 1996; Addo-Bediako *et al.* 2001; Chown 2002) were tested in **Chapter 4**. In addition, fluctuating temperatures are a predominant feature in any environment, including that of wandering albatross nests (Sinclair & Chown 2006). Theory predicts that fluctuating temperatures should increase metabolic rates due to metabolic rate being disproportionately higher during the warm periods than it is lower during the cool periods. Termed Jensen's inequality, when a high metabolic rate is costly, ectotherms may respond to fluctuating temperatures by lowering their thermal sensitivity (Ruel & Ayres 1999). These ideas were tested by acclimating caterpillars to both constant and fluctuating temperatures.
- A common assumption made by ecophysiologists is that preference will be for temperatures at which performance is maximised (i.e. coadaptation, see Angilletta *et al.* 2006). In **Chapter 5**, the relationship between thermal preference and locomotor performance of *P. marioni* caterpillars was explored. Specifically, how closely matched these traits are, and hypotheses that may explain the departure from coadaptation were tested. These are: (i) 'suboptimal is optimal', performance decreases rapidly once optimum temperatures are reached and preference should be for lower temperatures (Martin & Huey 2008); (ii) 'thermodynamic effects hypothesis', in the presence of a thermodynamic effect, natural selection should favour a thermal optimum that exceeds mean body temperature (Asbury & Angilletta 2010); (iii) 'trait variation', if optimum temperatures vary among physiological processes, then no single thermal preference will optimize all systems (Huey & Stevenson 1979); and (iv) the 'immobility hypothesis', preference will be low if organisms are trapped at the lower end of laboratory gradients.

- Two hypotheses were proposed to explain the high caterpillar biomass in wandering albatross nests. The 'nutrient hypothesis' as a consequence of high concentrations of nutrient availability in and around nests (Smith 1976; 1978; 1979; Joly *et al*. 1987), and more recently, the 'thermal ecosystem engineering hypothesis', owing to warmer nest temperatures that promote growth and survival of caterpillars (Sinclair & Chown 2006). Both these hypotheses assume that selection will have favoured nest location in either ovipositing moths or in caterpillars. Insects have been shown to use olfactory and thermal cues to choose suitable habitat and oviposition sites (Bruce *et al.* 2005; Anderson *et al.* 2013; Kleinedam *et al.* 2007; Clissold *et al.* 2013) and in **Chapter 6**, whether caterpillars use such cues to likely seek out nests was tested.
- Finally, **Chapter 7** draws together the outcomes of this work to better explain the role of wandering albatross in contributing to the high abundance of *P. marioni* caterpillars in nests.

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Chapter 2 – Solving the puzzle of *Pringleophaga* – threatened, keystone detritivores in the sub-Antarctic[1]



Pringleophaga marioni larva, pupa and adult moth.

Photo credit: C. Janion-Scheepers

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Abstract

In the globally significant, lowland terrestrial systems of the sub-Antarctic's South Indian Ocean Province (SIOP) Islands, caterpillars of the flightless moth genus *Pringleophaga* (Lepidoptera: Tineidae) are typically responsible for much nutrient turnover. On Marion Island, *P. marioni* is a keystone species for this reason. Rising temperatures have led to increasing populations of introduced house mice, which, in turn, feed extensively on *Pringleophaga* caterpillars. Because of the caterpillars' keystone role, predation by mice is leading to changes in the functioning of the terrestrial system. Given the estimates of an extended life cycle duration for *P. marioni* (and its congeners), i.e. two to more than five years, an ongoing puzzle is why the species has not shown greater population declines on Marion Island than have been recorded (in some habitats 40-97% in 20 years), given extremely high estimates of predation (*c*. 1 % of standing biomass per day). One reason may be inaccurate previous estimates of life cycle duration for the species. Here I provide a new, quantitative estimate, by rearing caterpillars at different temperature regimes (5, 10, 15 and 5-15°C), and combining these results with additional data from a prior study, to demonstrate that the life cycle duration for this species is approximately one year - half the previous minimum estimate. The new quantitative data provide grounds for improved models for estimating population persistence of this species, and information for models assessing the costs and benefits of conservation interventions such as the eradication of invasive house mice.

Keywords: Detritivore – house mouse – life cycle – null point of development – predation – sum of effective temperatures

Introduction

The South Indian Ocean Province (SIOP) Islands of the sub-Antarctic represent unusual ecosystems that have been accorded considerable conservation significance (de Villiers *et al*. 2005). On these islands terrestrial herbivory is uncommon, no indigenous mammalian herbivores are present, and even among the indigenous invertebrates, herbivory is infrequent (Holdgate 1977; Chown & Convey 2007). A monophagous lepidopteran and several weevil species, many of which are algae, lichen and moss feeders, constitute the majority of the insect herbivores (Chown 1994; Klok & Chown 1998; Vernon *et al*. 1998). In consequence, most primary productivity accumulates as peat, and nutrient recycling and mineralization largely take place via a detritus-based food web (Smith & Steenkamp 1990). Decomposition is facilitated by the activity of several invertebrate species, among the most significant of which are caterpillars of the flightless moth genus *Pringleophaga* (Lepidoptera: Tineidae), which are abundant on the Prince Edward, Crozet and Kerguelen archipelagos (three species: *P. crozetensis* Enderlein (Crozet Islands), *P. kerguelensis* Enderlein (Crozet and Kerguelen Islands), *P. marioni* Viette (Prince Edward Islands)) (Viette 1949; Crafford *et al*. 1986). Indeed, in the most comprehensively studied system, Marion Island of the Prince Edward group, caterpillars of *P. marioni* are responsible for substantial increases in nutrient mineralization (on average 17x rates found in their absence) and may process in excess of 100 $kg.ha^{-1}$ dry mass of litter per year (Smith & Steenkamp 1992; 1993). *Pringleophaga marioni* has therefore been identified as a keystone species, and this role likely extends to other islands given the abundance of *Pringleophaga* spp. there (see e.g. Joly *et al*. 1987).

Because of its keystone role, *P. marioni* has also been recognized as a significant component of the way in which interactions between climate change and biological invasions are affecting many of the SIOP islands (Smith & Steenkamp 1990; Bergstrom & Chown 1999). In brief, rising temperatures are leading to increasing populations of introduced house mice, which in turn take *Pringleophaga* caterpillars as preferred prey (Crafford & Scholtz 1987; Le Roux *et al*. 2002), and this is altering the functioning of local systems (Smith 2008). Indeed, at the Prince Edward Islands, where mice are found on Marion Island (MI), but not neighbouring Prince Edward Island (PEI), substantial differences in *Pringleophaga* population densities have been identified between the islands (Crafford & Scholtz 1987), and evidence exists of further declines in abundance of *P. marioni* on Marion Island (Chown *et al*. 2002). However, an ongoing puzzle is why *P. marioni* has not become exceptionally rare, especially since mice have alternative prey and therefore, in theory (Holt 1977), could potentially drive their preferred prey (Smith *et al*. 2002) to extinction. Much of the current

evidence suggests that at the very least *P. marioni* should be much more highly threatened by the invasive house mouse than appears to be the case (see e.g. Van Aarde *et al*. 2004): (i) Mice have been present on the islands since at least the 1800s (Cooper 2008). (ii) Estimates of their daily energy requirements, in combination with the past and present contribution of *P. marioni* caterpillars to their diet, indicate a daily consumption by mice of between 0.3 and 1.3% of caterpillar standing biomass (Rowe-Rowe *et al*. 1989; Smith *et al*. 2002), which is exceptionally high for insects generally (e.g. Cornell & Hawkins 1995; Hawkins *et al*. 1997; Zalucki *et al*. 2002). Although prey switching by mice has been found (Chown & Smith 1993), caterpillars continue to be found as a dominant item in mice gut contents (Smith *et al*. 2002; McClelland 2013). Moreover, habitat overlap between mice and caterpillars is virtually complete (e.g. Crafford & Scholtz 1987; Van Aarde & Jackson 2007), and mice will take prey as small as aphids (Smith *et al*. 2002), suggesting that caterpillars are available and preferred by mice (iii) Current estimates of the life cycle duration of *P. marioni* range from two to more than five years (Crafford *et al*. 1986; Crafford 1990a), suggesting that annual replacement might be exceptionally slow (Rowe-Rowe *et al*. 1989).

Given that mouse energy requirements on the island are similar to those estimated for other populations of the species (see Smith *et al*. 2002), and that the historic records of mouse presence on the island are verifiable (Cooper 2008), two key parameters that may help resolve the puzzle are estimates of caterpillar biomass and of the life cycle duration of the species. The former certainly indicate a decline in biomass and density over time in some habitats (between 40 and 97% decline in biomass over 20 years (1977 to 1997) depending on the habitat, Chown *et al*. 2002), but in others the change is less clear (see data presented by Burger 1978; Van Aarde *et al*. 2004). In consequence, at the heart of the puzzle, or perhaps more analytically, key to understanding likely ongoing impacts and the population persistence of *P. marioni*, is the life cycle duration of the species. Such information is significant for two primary conservation reasons. First, forecasts are for increasing impacts by mice as the climate continues to warm (Smith & Steenkamp 1990). Although caterpillar development rate will likely increase and life cycle duration decline with increasing temperature, so potentially mitigating some predation impacts, the species is susceptible to high temperatures as we show here (see Results and Discussion). Population modelling to estimate the extent of the likely impact requires information on the life cycle of the species. Second, owing to impacts by mice on birds such as albatrosses (Jones & Ryan 2010), an eradication programme for these rodents is being contemplated given the efficacy of such interventions elsewhere (e.g. Howald *et al*. 2007). For comprehensive assessments of the

impacts of such programmes population level information on all components of the food web is required (Bergstrom *et al*. 2009; Raymond *et al*. 2011). Thus, resolving the duration of the life cycle of *P. marioni* will not only address a long-standing question about the biology of this species, but will also assist with conservation assessments of the outcomes of rodent eradication and its alternatives (the most likely of which is no action, given the costs of such programmes and that most programmes are focussed on total eradication). Here, we use new laboratory data and previously collected field data to do so.

Materials and methods

Because of the known recalcitrance of the species in the laboratory (Crafford 1990b), and the scarcity of adult female moths (Chown *et al*. 2002), first larval instar caterpillars, i.e. ~ 0.01 – 0.03 g (Crafford 1990b) were collected from abandoned wandering albatross (*Diomedea exulans*) nests (< 20) and tussock vegetation (*c*. 1 ha) near the research station on Marion Island (46°54'S, 37°45'E) in 2009. The caterpillars were visually identified as first instar based on their small size and this was verified by mass (as above) in the laboratory using a Mettler AE163 balance (Mettler Toledo, Sandton, RSA). A first collection was made in July and this was supplemented by a second collection in November. Caterpillars were randomly assigned to controlled temperature chambers (MIR 154, Sanyo, Osaka, Japan, accurate to ± 0.5°C) set at 5°C, 10°C, 15°C and 5-15°C (12:12 h, low temperature coinciding ~ scotophase) (n = *c*. 100 per temperature given the difficulty of rearing the species). Photoperiods were established according to the seasonal schedule of Marion Island: i.e. 16L: 8D for November, December, January; 12L: 12D for February, March, April, August, September and October; 8L: 16D for May, June, July. Individuals were held separately in Petri dishes filled with homogenized, moistened material from recently abandoned wandering albatross nests that served as both a food source and shelter. Keeping individuals separate was necessary to avoid cannibalism (French & Smith 1983). Relative humidity within such petri dishes is *c*. 100%. Caterpillars were provided with a fresh supply of nest material every week and the shelf location of batches of five or less petri dishes were re-arranged once a week to avoid shelf effects. The time at which an individual pupated, and if it emerged as an adult was recorded. Most of the trial was conducted at Marion Island, but the latter portion thereof (*c*. 3 months) was completed in South Africa (following return of the caterpillars in their incubators via ship to Stellenbosch, South Africa, with their conditions maintained unchanged throughout).

Generalized linear models (Quasipoisson distribution of errors, because of count data and overdispersion (see Crawley 2007), as implemented in R2.12.0. (R Core Team 2009)) were used to investigate the influence of temperature treatment on duration of the larval and pupal stages. Although mortality at 15°C was high, estimates of the lower development threshold (LDT) and sum of effective temperatures (SET) (see Honěk 1996) were made using individual data and ordinary least squares regression implemented in R.

To obtain additional information, especially on the duration of the pupal and adult stages, the number of eggs laid per female moth, and the extent of size variation in the field, previously unpublished data from Crafford (1990b) were also used. In that study, caterpillars of a variety of instars (> 50 per instar) were collected in the field (between 1983 and 1984), from which *c*. 20 adult moths were obtained. Both caterpillars and adults were maintained in the laboratory either at 5°C or at 10°C in custom-built incubators (described in Klok & Chown 1997) on a 12:12 L:D cycle. An earlier sampling programme, consisting of the collection of 100, 80 mm diameter cores per month, for a full year from May 1983 to April 1984, from the mire vegetation type (described in Crafford & Scholtz 1987) also provided caterpillars, which were then subject to head capsule (greatest width in dorsal perspective) measurements using a calibrated ocular micrometer fitted to a Wild M5 stereo microscope (Wild, Heerbrugg, Switzerland), to determine whether the progression of a cohort of individuals in the field could be identified. Here, a runs test (implemented in MS-Excel) was undertaken on the monthly data of the proportional abundance of sampled caterpillars with head capsule widths < 1 mm, to determine whether new cohorts appear at random, or whether a run of small, likely first instar (see Crafford 1990b), caterpillars was sampled.

Results and discussion

In the more recent laboratory study, *Pringleophaga marioni* larvae took approximately 46 to 66 weeks to complete their development at 10°C and 5°C, respectively (Fig. 1). Bearing in mind that laboratory trials commenced with first instar larvae, but that in Lepidoptera this instar is short compared with the others (see e.g. Scoble 1992; Davidowitz *et al*. 2004), it appears that development is completed at these temperatures in approximately a year. At 15°C, development was much faster, but mortality was high. Indeed survival in the laboratory was less than 5%, and this has been found in other investigations (T.M. Haupt, B.J. Sinclair, S.L. Chown, unpublished data). Survival was higher at the fluctuating temperatures, and development time was not significantly different to that at 10°C (compared using a stepwise deletion procedure, see Crawley 2007: 374, also Fig. 1). Bearing in mind high mortality at

15°C, the LDT and SET estimates were 0.8°C and 272 degree days, respectively. The former is in keeping with findings that the growth of *P. marioni* caterpillars is compromised by long-term exposure to sub-lethal low temperatures, even though they are freezing tolerant (Sinclair & Chown 2005).

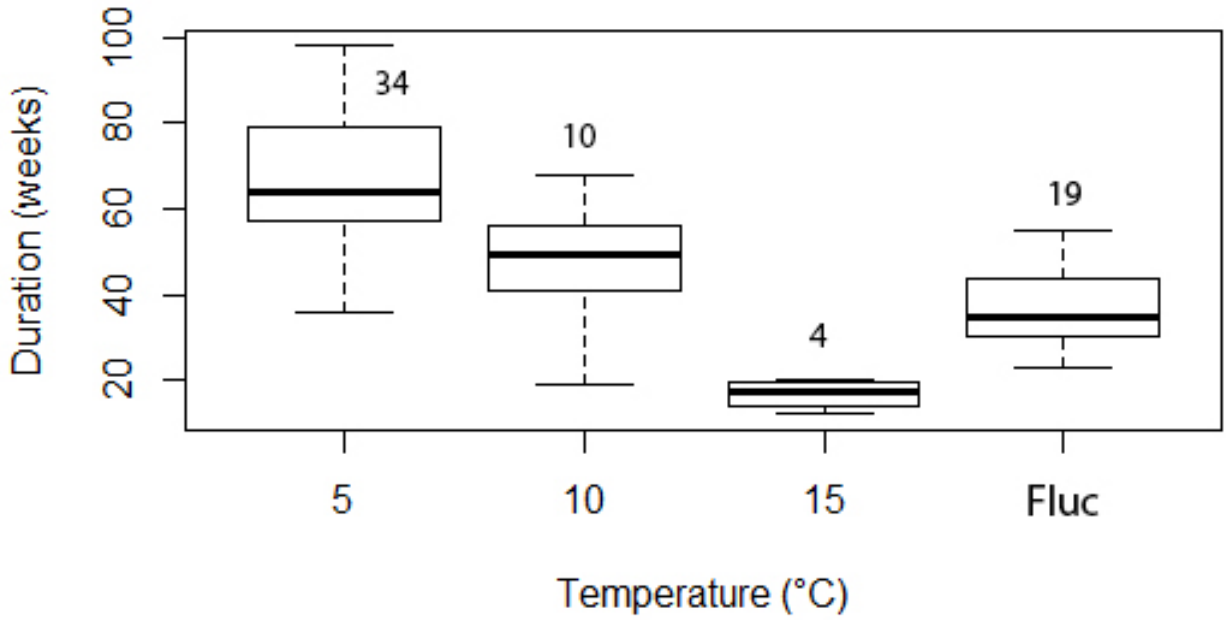

Fig. 1. Duration of the larval stage of *Pringleophaga marioni* at 5°C, 10°C, 15°C and a fluctuating temperature of 5-15°C (Fluc). Sample sizes are indicated above each of the box and whisker plots which indicate the median, quartiles and extremes. Means and standard errors for these data are: 66.7±14.0 (5°C), 46.0±15.5 (10°C), 16.8±3.6 (15°C) and 37.1±9.8 (fluctuating) weeks. Significant differences were found between the different temperature treatments except for 10°C and the fluctuating treatment (Generalized Linear Model, Quasipoisson distribution of errors, $t = 3.6$ to 13.2, $p < 0.0008$ in all significant cases).

Pupal duration documented by Crafford (1990b) was 52-65 days, with adult females living for 7-11 days and adult males 12-18 days. Females laid 203 eggs with an egg duration of 30-50 days. Soil microclimate recordings from Marion Island, taken at *c*. 50 m a.s.l, indicate that habitat temperatures vary from 3.8°C (mean minimum) to 8.6°C (mean maximum) (Deere *et al*. 2006). Summing the duration of the egg, larval and pupal stages, and bearing in mind this temperature range, suggests that *P. marioni* completes its life cycle

within one year. Considering that conditions in the field from a nutritional choice perspective (see Simpson & Raubenheimer 2012) will be more optimal than in the laboratory, and that caterpillars may seek out thermally optimal environments (Sinclair & Chown 2006), a year-long life cycle is plausible. Head capsule measurements from the 1983-1984 field survey also suggest that cohorts of individuals with a *c*. 1 year interval are present (Fig. 2). In particular, the runs test on individuals with head capsule widths < 1.0 mm indicated that the data are non-random ($Z = -2.422$, $p < 0.016$ (two-tailed)). As a proportion of the total sample for each month, the months of January to June have fewer than 10% of individuals of this size, whereas between July and December the proportion exceeds 10%, peaking at 25% in October, in keeping with a general increase in adult insect activity on the island in early spring (e.g. Chown & Scholtz 1989).

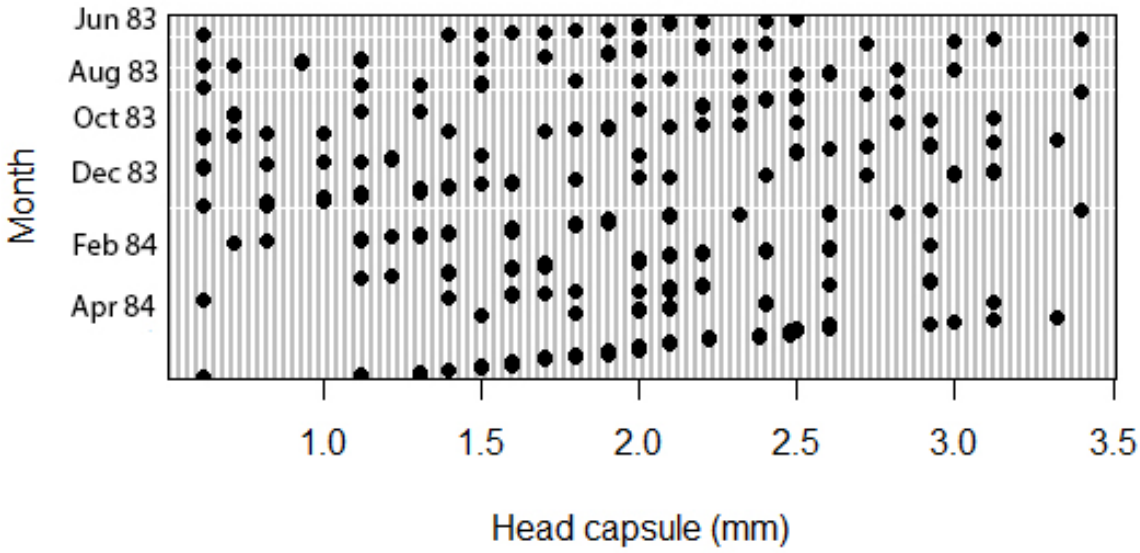

Fig. 2. Cleveland dotplot of head capsule measurements of *Pringleophaga marioni* caterpillars collected in a mire plant community between 1983 and 1984. Dots indicate single individuals. Note the scarcity of first instars in the late austral summer and early winter.

A year-long life cycle for *P. marioni* is at least half as long as that previously estimated (Crafford *et al*. 1986) and may go some way to explaining why the species has not declined to very low numbers on Marion Island. That is, population replacement is faster than has previously been assumed. The finding does not mean, however, that *P. marioni* is not at

substantial risk from mouse predation, given that it remains a preferred prey species (Smith *et al*. 2002) and census estimates indicate a declining population (Chown *et al*. 2002). Nonetheless, the new estimate of life cycle duration will make formal models of population persistence more accurate, and will also facilitate modelling of the outcome of various possible conservation interventions (from none to full eradication of mice). In the former case, and given the restricted distribution of the species (only Marion and Prince Edward Islands), the potential now exists for formal assessment of the species according to the IUCN red list criteria. To date, no such assessments have been made for plants or invertebrates in the sub-Antarctic.

The information provided here will also contribute to improved understanding of the genetic structure of the species on the island (and the two *Pringleophaga* species found on the other islands, see Viette 1949; Paulian 1953) because of better estimates of divergence times among locations (see Groenewald *et al*. 2011), which depend on estimates of life cycle length. The current findings also contextualize the species in the polar, and especially the sub-Antarctic, insect fauna more generally (Vernon *et al*. 1998). That is, they have a year-long life cycle, as do many other sub-Antarctic species, such as the ectemnorhine weevils (Chown 1994), but they have a relatively brief adult stage. This makes them most similar to the 'Category 1' species with long life cycles discussed by Danks (1992). Although a year-long life cycle is relatively compressed compared with insects such as the 17-year cicada, some wood feeding beetle species, and several other polar taxa (Danks 1992; Convey 1996), it is still much longer than is typical for most insects (Fig. 3).

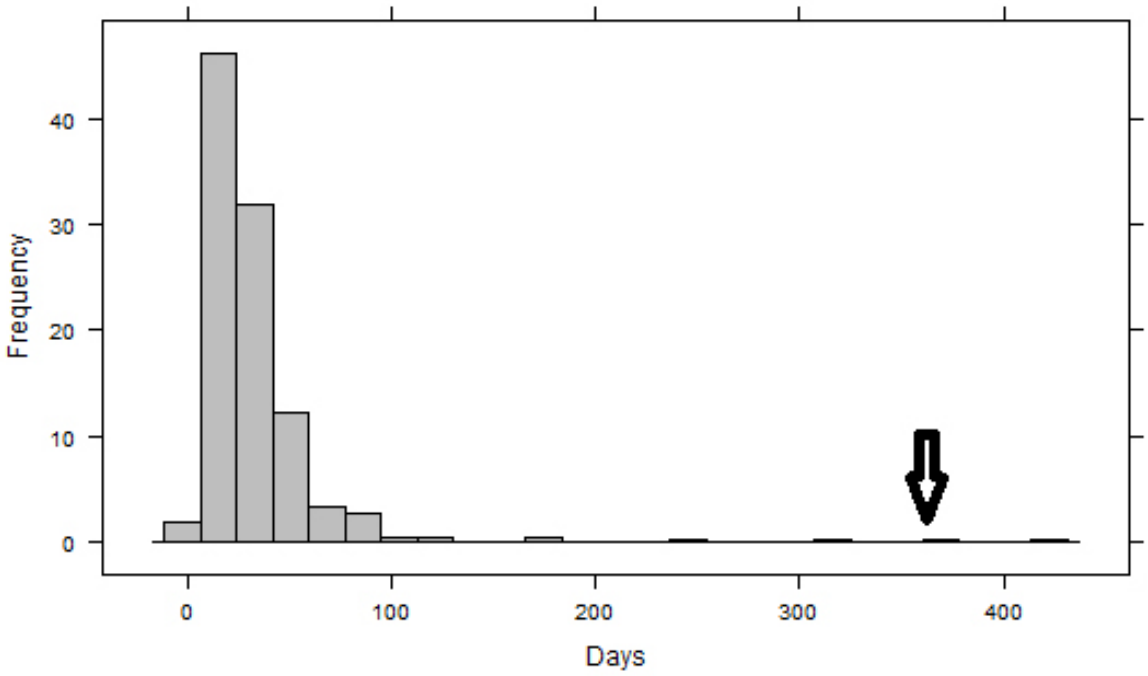

Fig. 3. Histogram of development times (days) of insects at their average habitat temperature (typically 20-25°C) from the data compiled by Irlich *et al*. (2009) (here n = 501 and frequency is %), indicating the location of *P. marioni* at 10°C (arrow). The record life cycle duration for insects (not shown on the histogram) is *c*. 17 years or > 6200 days.

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Chapter 3 – The nests of the wandering albatross: a further investigation of caterpillar biomass and temperature

Researchers searching for caterpillars in a wandering albatross nest on Marion Island.

Photo credit: B. J. Sinclair

Abstract

Wandering albatross (*Diomedea exulans*) on Southern Ocean Islands build elevated nests upon which they incubate eggs and raise chicks. These nests support high invertebrate biomass, including larvae of flightless moth caterpillars, *Pringleophaga marioni.* Previous investigations suggested that the high densities of invertebrates found within albatross nests are a consequence of nutrient availability around and in nests. Subsequently, it has also been suggested that nutrients may not be responsible for the high densities of *P. marioni* found in wandering albatross nests on sub-Antarctic Marion Island as nutrient concentrations were found to be either similar or lower than that of other island habitats. Rather, since incubating birds raise nest temperatures by 5°C compared to surrounding soils, a role for temperature was proposed. Given that caterpillars are susceptible to repeated low temperature stresses, it was hypothesised that wandering albatrosses may serve as thermal ecosystem engineers by providing a warm microhabit in which growth and survival may be improved. This hypothesis presented by Sinclair & Chown (2006) is a compelling one, however, the caterpillar abundance sampling undertaken was relatively restricted to old and recently failed nests (new). Nests that were occupied the previous season, and from which chicks had fledged, were not sampled. Moreover, in the prior study, temperature data were collected over a short period of time whereas nests are occupied for an entire year. In this study, my primary goals were to examine caterpillar abundance in all three nest types, i.e. old, fledged and new, and to conduct a more extensive sampling effort of temperatures within occupied nests and other habitats over the duration of the nesting period. Results showed that caterpillar abundance was significantly higher in new nests compared to old and fledged nests. Moreover, the low variation in caterpillar biomass in new nests compared to other vegetation complexes suggest that caterpillars were distributed more evenly within new nests than any other habitat on Marion Island. During the full duration of nest occupancy, the temperatures within occupied nests were also significantly higher compared to surrounding soils and abandoned nests. In particular, daily minimum and absolute minimum temperatures were significantly higher in occupied nests compared to all other habitats and temperature events at which *P. marioni* experience chill coma were substantially reduced.

Keywords: sub-Antarctic – wandering albatross nests - ecosystem engineering – invertebrate abundance - temperature

Introduction

Despite the ease with which boundaries to ecosystems can be described, such as between above or below-ground systems, or between the marine and terrestrial realms, all ecosystems are semi-open. Between the land-sea interface, nutrients and energy may enter the ocean both directly, and also indirectly via rivers and estuaries (Barnes & Hughes 1988; Balls 1994; Jickells *et al.* 2000), resulting in high primary and secondary marine productivity (Martin *et al.* 1989; Lohrenz *et al.* 1999; McKee *et al*. 2004; Schlacher *et al.* 2009). Similarly, terrestrial ecosystems may be greatly enriched by marine derived nutrients which are transferred via aerosols, shore drift of algae and carrion, marine mammals, and also seabirds (Smith 1978; Polis & Hurd 1996; Polis *et al.* 2004; Bokhorst *et al.* 2007). Seabirds in particular, are significant mediators of these interactions and their primary role is in the transfer of energy and nutrients when they come ashore to breed or to moult (Williams & Berruti 1978; Harding *et al.* 2004). During these times, energy and material flows can be significant and are typically through prey remains (Williams *et al.* 1978), feathers (Williams & Berruti 1978), eggshells (Siegfried *et al.* 1978) and importantly, guano (Hutchinson 1950; Burger *et al.* 1978; Bosman & Hockey 1988; Wootton 1991; Wainright *et al.* 1998; Wait *et al.* 2005). The significance of seabirds in transferring nutrients from the oceans to the land is widely recognized (Heatwole 1971; Mizutani & Wada 1988; Polis & Hurd 1996; reviews in Polis *et al.* 1997 and Ellis 2005), and they are instrumental in influencing terrestrial dynamics in a range of systems (e.g. Polis & Hurd 1995; Anderson & Polis 1999; Stapp *et al.* 1999; Sánchez-piñero & Polis 2000; Keatley *et al.* 2009; Griffiths *et al.* 2010; Kolb *et al.* 2012).

Seabirds also influence terrestrial systems in other ways, particularly as ecosystem engineers. The latter are organisms that modify resources available to other species by either their presence or actions, and in so doing create, modify or maintain habitats (Jones *et al.* 1994; 1997; Wright & Jones 2006; Cuddington *et al.* 2007; Hastings *et al.* 2007). Beaver dams have become the iconic example of ecosystem engineering (Naiman *et al.* 1986; Wright *et al.* 2002). Seabirds make their ecosystem engineering contribution largely through the excavation of burrows and the construction of other forms of nests. These may result in the displacement of tons of material, creating new habitats for a range of other organisms (Joly *et al.* 1987; Newman 1987; Bancroft *et al.* 2004; 2005; McKechnie 2006; Moravcová *et al.* 2010; but see Mulder & Keall 2001 and Vincke *et al.* 2007).

On the Southern Ocean Islands, seabirds are important in transferring nutrients from the sea to terrestrial systems (Smith 1976; 1978; 1979; Burger *et al.* 1978; Williams & Berruti 1978; Williams *et al.* 1978; Siegfried 1981; Erskine *et al.* 1998; Vidal *et al.* 2003). One of the most extensive quantifications of this transfer has come from work done on sub-Antarctic Marion Island as part of the International Biological Program (IBP), and was recently summarized, along with more current work (Smith 2008; Smith & Froneman 2008). From small, burrowing petrels to the larger penguins and albatrosses, all surface and sub-surface nesters make significant nutrient transfers, with further influences on the invertebrate biota and ecosystem functioning (Smith 1976; 1978; 1979; Crafford & Scholtz 1986; Gabriel *et al.* 2001; Barendse *et al*. 2002).

More recently, Sinclair & Chown (2006) suggested that wandering albatrosses, *Diomedea exulans*, also act as thermal ecosystem engineers on Marion Island. Specifically, the biomass of flightless tineid moth caterpillars, *Pringleophaga marioni*, are generally highest in nests compared to other habitats on the Island, and also higher in new nests (recently failed nests were the proxy for these) compared to old nests. In addition, caterpillars are distributed more evenly in nests than in other habitats (Sinclair & Chown 2006). Sinclair & Chown (2006) found that nutrient availability was not responsible for this high biomass (e.g. Joly *et al.* 1987). Rather, it was suggested that, since incubating birds raise nest temperatures to five degrees higher than the surrounding soils (Sinclair & Chown 2006), this may favour caterpillar growth and survival. These warmer microhabitats are close to the optimum temperature for caterpillar feeding (Crafford 1990), and may also favour *P. marioni*, as caterpillars are susceptible to repeated low temperature stresses (Sinclair & Chown 2005). From these findings, it was suggested that wandering albatrosses may be serving as ecosystem engineers to invertebrates by creating a more favourable microhabitat with thermal benefits (Sinclair & Chown 2006).

Although the evidence presented by Sinclair & Chown (2006) in favour of the thermal ecosystem engineering hypothesis seems compelling, the caterpillar abundance sampling undertaken was restricted to old and recently abandoned nests (new nests), whereas nests that were occupied the previous season, and from which chicks had fledged, were not sampled. These nests might either be expected to have relatively high numbers of caterpillars, or low numbers, depending on whether or not caterpillars or moths seek out nests and at what point in the nest duration this is done (see Chapter 6). One goal of this study was therefore to conduct a more extensive sampling effort of caterpillar biomass in all three nest types, i.e.

old, fledged and new. Moreover, in the prior study, temperature data were collected over a period of two weeks, whereas nests are occupied for a full year (Ryan & Bester 2008). In the current study, temperatures within nests (a different set from the above) and surrounding habitats are assessed over a full year. Combined, these data may allow for a better understanding of the extent to which wandering albatross might serve as thermal ecosystem engineers, and also some of the possible mechanisms which may account for the way this engineering may take place. This was achieved by examining the effect of nest age (i.e. old, new and fledged nests) on caterpillar abundance and also by deploying miniature temperature data loggers for most of the nesting duration in occupied nests, abandoned nests, as well as soils adjacent to these nests.

Materials and methods

Study site and species

Marion Island (46°54'S, 37°45'E) is part of the Prince Edward Island group, and as is typical of sub-Antarctic Islands, the climate is cool, wet and windy (Smith 2002). The Prince Edward Islands support some 44% of the global wandering albatross population (Underhill *et al.* 2003). Their nests are large mounds of vegetation and peat collected from the surrounding area. A single egg is laid in mid-summer, hatches in March, and chicks fledge anytime between December and February (Ryan and Bester 2008). Nests are therefore occupied for almost a whole year. The nest inquiline, *P. marioni* caterpillars, are endemic to the Prince Edward Islands, and apart from wandering albatross nests, caterpillars or adult moths are found in a range of habitats from saltspray plant communities on the coast, to microhabitats under stones and on moss and cushion plants at altitudes up to 800 m (Crafford 1990; Smith & Steenkamp 1992). The physiology of this species is well known, caterpillars have a critical thermal minimum (CTmin) temperature range of -1.6°C to 0.1°C, a supercooling range (i.e. temperature at which caterpillars freeze) between -3.4°C and -7.1°C and a lower lethal temperature (i.e. 100 % mortality) of -9°C (Klok & Chown 1997). The caterpillars are moderately freeze tolerant, but do experience low temperature-related reductions in growth rate (Klok & Chown 1997; Sinclair & Chown 2005; Haupt *et al.* 2013).

Sampling of nests and other habitats for caterpillars

During the relief voyage of April/May 2012, 43 abandoned wandering albatross nests were identified between the meteorological station on Marion Island's east coast up to Ship's Cove and Macaroni Bay located to the west and east respectively (Fig. 1).

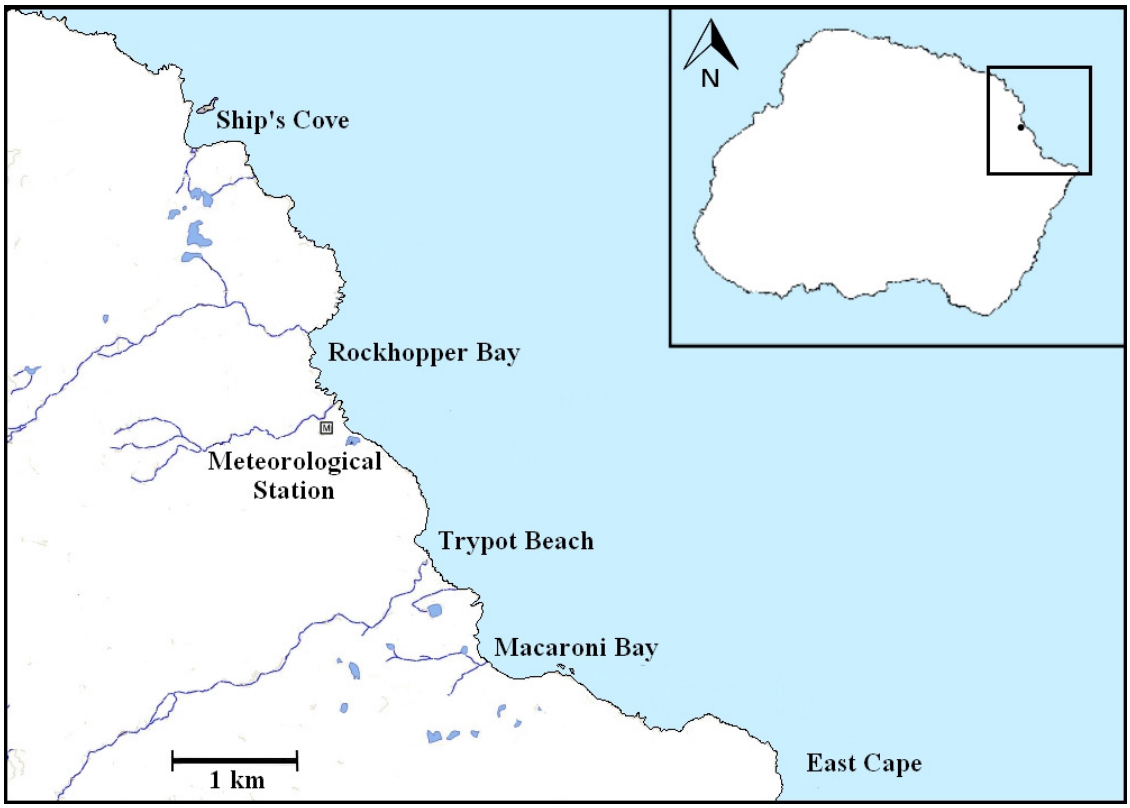

Fig. 1. The areas in which abandoned wandering albatross nests were sampled along Marion Island's East coast between the meteorological station up to Ship's Cove and Macaroni Bay.

Nests were located mainly in dry or wet mire communities, or along *Blechnum penna-marina* slopes (Gremmen 1981). Following Sinclair & Chown (2006) only nests not currently occupied by birds were sampled. These were termed 'abandoned nests' and subsequently grouped into three types – old, fledged and new. These categories build on those used by Chown & Sinclair (2006), recognizing that nests which have not been recently abandoned may have had a chick the previous season or may not have. Researchers working over the 2011/2012 field season provided this information based on nest monitoring routinely conducted at the island (Crawford & Cooper 2003). The three categories of nests can best be

summarized as follows: 1) old, which were nests that had not been occupied for most of 2011 or the previous seasons, 2) fledged, which were nests that had been occupied the previous season, i.e. built in November 2010 and from which chicks had fledged between December 2011 and January 2012, and 3) new, which were nests built for the current season, i.e. in November 2011, but had recently failed due to egg or chick mortality. New nests were easily identified by tufts of vegetation scattered in the vicinity, an indication of recent nest building activities, and by the absence of vegetation on the nests. New nests often had remnants of egg shells, feather down and sometimes remains of a dead chick could be found. Old and fledged nests were almost always overgrown with vegetation and trampled flat. Fifteen new nests and fourteen of each of the fledged and old nests were sampled. These nests were selected haphazardly across the sampling area (Fig. 1). Additional information was then recorded following the methods of Sinclair & Chown (2006). At each nest, the location was taken using a global positioning system (GPS), the height and diameter measured (cm) with a tape measure, and a record made of vegetation occurring on and surrounding the nest, as well as the number of mouse burrows. Nests are ellipsoid, thus, two diameter measurements were recorded to obtain the full surface area of each nest. The distance from nutrient rich sources such as the coast, animal communities, and *Poa cookii* tussocks were also noted. The nest material was searched by hand for caterpillars by a number of samplers. This search effort involved complete dismantling of the nest and careful searching of all of the material. No time limit was set on the searches and typically a nest search was completed in 1-2 hours. Caterpillars were placed in 350 ml plastic jars filled with nest material and returned to the laboratory within six hours after collection where individuals were counted and weighed (g) wet using a Mettler AE163 balance (Mettler-Toledo Ltd, EngNet, South Africa). After weighing, caterpillars were returned within 24 h to the nests from which they were collected. Individual caterpillar dry mass was obtained from a linear regression of dry on wet mass by using the mass of 138 individuals dried at 60°C from a prior study on caterpillar physiology (Sinclair *et al.* 2005) (dry mass = 0.113311 x wet mass (g) (intercept = 0)). Since caterpillars are usually concentrated in the surface layers of the nest (T.M. Haupt, S.L. Chown, personal observations), the surface area of each nest (m^{-2}) was calculated as opposed to the entire volume. Surface area was calculated per the area of an ellipse:

$$A = \pi \text{ x r1 x r2},$$

where “r1” and “r2” are half the lengths of the largest and smallest diameters respectively. Caterpillar biomass and density in nests was expressed as mg m^{-2} and numbers m^{-2} respectively.

Estimates of caterpillar biomass and density in nests were compared with those obtained from invertebrate surveys in April and May of 1997 (Hänel 1999). These months were chosen as nests were also sampled during this time period in the current study. Five vegetation types were compared and these were - mire communities: *Sanionia uncinata* and *Blepharidophyllum densifolium*, and non-mires: *Poa cookii*, *Cotula plumosa* and *Crassula moschata*. In each of these complexes, five 2 x 2 m quadrants were staked out at random, and from each of these, two soil cores (7 cm diameter) were extracted every alternate month. Cores were sorted through by hand in the laboratory where the sample was first sorted dry, after which it was washed to remove any remaining invertebrates. Caterpillars and other invertebrates were then identified, weighed wet, dried to a constant mass at 60°C, and then weighed dry (Hänel 1999). Since invertebrates are usually only found in the top cm of soil (S.L. Chown, personal observation), the surface area of the cores (m^{-2}) were calculated as per the area of a circle:

$$A = \pi r^2,$$

where “r” is equal to the radius. Caterpillar biomass and density from cores was expressed as mg m^{-2} and numbers m^{-2} respectively.

Microclimate measurements

Temperature

During the April-May relief voyage in 2011, ten occupied and ten abandoned wandering albatross nests were identified around the meteorological station and along the route to Macaroni Bay on the East coast (Fig. 1). Two calibrated thermocron iButtons (Model DS1922L, Maxim Integrated, Fairbridge, South Africa) were inserted into each nest. These were set to record at hourly intervals from May 2011 to March 2012. iButtons were attached to plastic cable-ties to make relocation possible and were inserted mid-height on the side of each nest. Care was taken not to damage the nest structure in any way. This was particularly important for occupied nests, and for this reason, experienced bird handlers were present

when inserting iButtons into these nests. Soil temperature nearby each nest was measured for the same time period with iButtons (Model DS1922L, Models DS1922L, Maxim Integrated, Fairbridge, South Africa) that were inserted in the side of plastic marker poles and placed below the soil 2 m away from each nest. These were also set to record temperature at hourly intervals. For occupied nests, a record was made by overwintering researchers monitoring the wandering albatross population in the instance of chick mortality or fledgling date.

Statistical analyses

Caterpillar abundance (i.e. biomass and density) between nest types (old, fledged and new) was compared using a generalized linear model (negative binomial distribution of errors, log link function because of count data). Caterpillar biomass and density between new nests and vegetation complexes were compared using a Hurdle model (because of zero-inflated data, negative binomial distribution because of overdispersion). The latter model may be applied when many zeros are present in the data (Zuur 2009), as was the case when caterpillars were absent in quadrants of the vegetation complexes sampled.

Microclimate temperature data from occupied and abandoned nests, as well as adjacent soil habitats were divided into months, and for each month the mean, daily minimum and maximum, and absolute minimum and maximum temperatures were obtained. For each month, a generalized linear model (Gaussian distribution of errors, log-link in the case of absolute minimum and maximum as data were skewed) was used to compare temperature traits of occupied nests with abandoned nest and soil habitats. For each nest and adjacent soil habitat, temperatures at which caterpillars are below their CTmin, i.e. < 0.2°C (because of the CTmin range of -1.6°C to 0.1°C), supercooling point, i.e. < -3.3°C (because of the supercooling range of -3.4°C to -7.1°C), and lower lethal temperature of < -8.9°C (because of 100 % mortality at -9°C respectively), were counted in Excel (Microsoft 2010, Version 14). All analyses were implemented in R3.0.0 (R core team, 2013).

Results

Caterpillar biomass and density in new nests (Fig. 2) was significantly higher than in old and fledged nests (Tables 1 and 2). Moreover, the coefficient of variation of caterpillar biomass in

new nests was lower than that found for fledged and old nests (Fig. 2). Compared to vegetation complexes sampled in April and May 1997 (Hänel 1999), caterpillar biomass in new nests was significantly lower than in *Sanionia uncinata* and *Cotula plumosa*, no different than in *Crassula moschata*, and significantly higher than in *Blepharidophyllum densifolium* and *Poa cookii* (Table 3). Caterpillar density in new nests was significantly lower compared to *S. uncinata*, *C. plumosa* and *B. densifolium*, and no different to that found in *C. moschata* and *P. cookii* (Table 4). By contrast, the coefficient of variation in nests, and new nests in particular, was lower, in the latter case by 5-6x, than in all other habitats (Fig. 2).

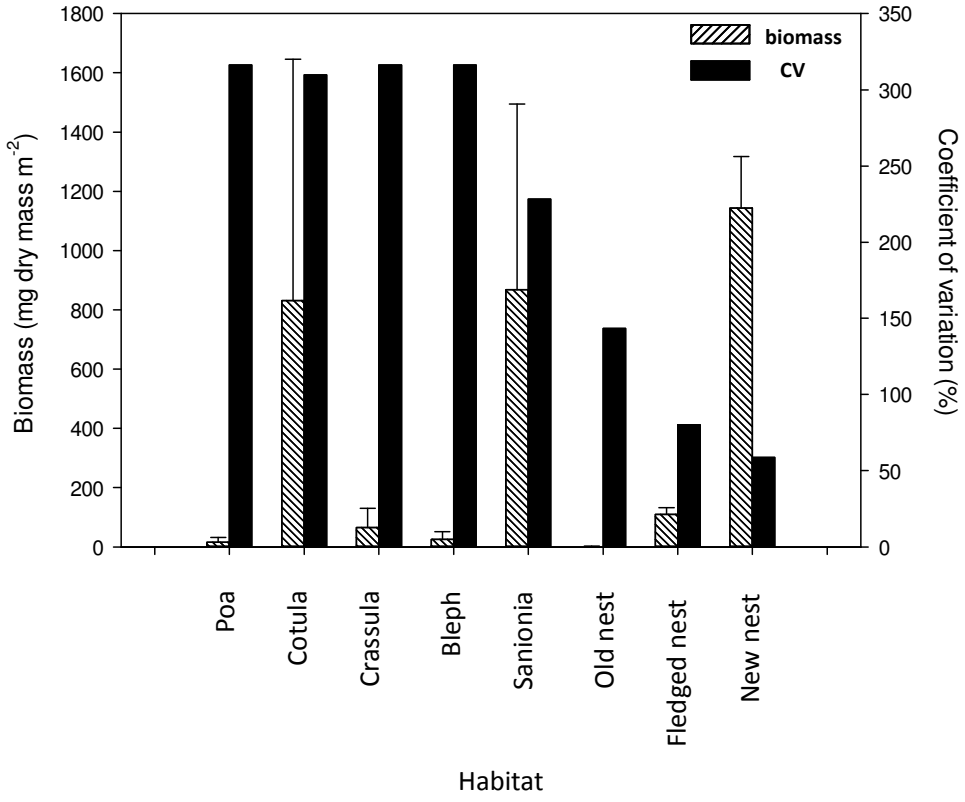

Fig. 2. Mean (± SE) and coefficient of variation (CV) of biomass (mg m^{-2}) of *Pringleophaga marioni* caterpillars in eight habitat types on Marion Island: five vegetation complexes sampled by Hänel (1999) in April or May 1997 (*Poa cookii*, *Cotula plumosa*, *Crassula moschata*, *Blepharidophyllum densifolium* and *Sanionia uncinata*), and old, fledged and new nests sampled in this study.

Table 1. Outcome of a generalized linear model (negative binomial distribution of errors, log link function) comparing caterpillar biomass (mg m^{-2}) in new nests vs. old and fledged nests.

	Estimate	s.e.	*z*	*P*
intercept	**7.042**	**0.194**	**36.238**	**< 0.0001**
habitat (fledged nest)	**-2.350**	**0.281**	**-8.371**	**< 0.0001**
habitat (old nest)	**-6.637**	**0.355**	**-18.713**	**< 0.0001**

s.e. = standard error, bold indicates significant differences

Table 2. Outcome of a generalized linear model (negative binomial distribution of errors, log link function) comparing caterpillar density (numbers m^{-2}) in new nests vs. old and fledged nests.

	Estimate	s.e.	*z*	*P*
intercept	**4.653**	**0.167**	**27.812**	**< 0.0001**
habitat (fledged nest)	**-2.236**	**0.252**	**-8.861**	**< 0.0001**
habitat (old nest)	**-7.292**	**1.028**	**-7.092**	**< 0.0001**

s.e. = standard error, bold indicates significant differences

Table 3. Outcome of the hurdle model (with negative binomial distribution of errors), comparing caterpillar biomass (mg m^{-2}) in new nests with that found in five vegetation complexes (*Blepharidophyllum densifolium*, *Cotula plumosa* and *Crassula moschata*, *Poa cookii* and *Sanionia uncinata*) in April and May 1997 (Hänel 1999)).

Count model coefficients (truncated negative binomial with log link):				
	Estimate	s.e.	*z*	*P*
intercept	**7.042**	**0.184**	**38.249**	**< 0.0001**
habitat (*B. densifolium*)	**-1.485**	**0.739**	**-2.011**	**0.0443**
habitat (*C. plumosa*)	**1.291**	**0.537**	**2.406**	**0.0161**
habitat (*C. moschata*)	-0.565	0.737	-0.767	0.4432
habitat (*P. cookii*)	**-1.992**	**0.740**	**-2.691**	**0.0071**
habitat (*S. uncinata*)	**1.333**	**0.537**	**2.485**	**0.0129**
Zero hurdle model coefficients (binomial with logit link):				
	Estimate	s.e.	*z*	*P*
intercept	19.570	4577.960	0.004	0.9970
habitat (*B. densifolium*)	-21.760	4577.960	-0.005	0.9960
habitat (*C. plumosa*)	-20.950	4577.960	-0.005	0.9960
habitat (*C. moschata*)	-21.760	4577.960	-0.005	0.9960
habitat (*P. cookii*)	-21.760	4577.960	-0.005	0.9960
habitat (*S. uncinata*)	-20.950	4577.960	-0.005	0.9960

s.e. = standard error, bold indicates significant differences

Table 4. Outcome of the hurdle model (with negative binomial distribution of errors), comparing caterpillar density (numbers m^{-2}) in new nests with that found in five vegetation complexes (*Blepharidophyllum densifolium*, *Cotula plumosa* and *Crassula moschata*, *Poa cookii* and *Sanionia uncinata*) in April and May 1997 (Hänel 1999)).

Count model coefficients (truncated negative binomial with log link):				
	Estimate	s.e.	*z*	*P*
intercept	**4.653**	**0.142**	**32.848**	**< 0.0001**
habitat (*B. densifolium*)	**2.986**	**0.559**	**5.346**	**< 0.0001**
habitat (*C. plumosa*)	**1.313**	**0.409**	**3.212**	**0.0013**
habitat (*C. moschata*)	0.907	0.562	1.616	0.1062
habitat (*P. cookii*)	0.907	0.562	1.616	0.1062
habitat (*S. uncinata*)	**0.907**	**0.410**	**2.216**	**0.0267**
Zero hurdle model coefficients (binomial with logit link):				
	Estimate	s.e.	*z*	*P*
intercept	19.570	4577.960	-0.004	0.9970
habitat (*B. densifolium*)	-21.760	4577.960	-0.005	0.9960
habitat (*C. plumosa*)	-20.950	4577.960	-0.005	0.9960
habitat (*C. moschata*)	-21.760	4577.960	-0.005	0.9960
habitat (*P. cookii*)	-21.760	4577.960	-0.005	0.9960
habitat (*S. uncinata*)	-20.950	4577.960	-0.005	0.9960
s.e. = standard error, bold indicates significant differences				

Because some iButtons either failed to record, were irrecoverable, or chicks had died soon after sampling of nest temperatures began (Fig. S1), temperature data were only available for three occupied nests, eight soil habitats adjacent to occupied nests, five abandoned nests, and five soil habitats adjacent to abandoned nests. Temperatures in occupied nests were higher than in other habitats (Fig. 3) with a broad range of daily minimum and maximum temperature fluctuations (Table 5). Overwintering researchers observed that the chicks had begun venturing away from the nests at the beginning of December and fledged at the end of the month. The decline in temperature of the now vacant nests was noticeable at this point (Fig. 3). Whilst nests were occupied (May – December

2011) monthly temperature parameters (i.e. mean, daily minimum and maximum, and absolute minimum and maximum) were mostly significantly higher in occupied nests compared to adjacent soils and also abandoned nests and adjacent soils (Table 5; Table 6). After chicks had fledged (i.e. ~ December 2011 and January 2012), there were mostly no significant differences in the monthly temperature parameters between these habitats (Table 6). When significant differences occurred, they were with abandoned nests or soil adjacent to these nests, and this may be because of the different location of nest sites.

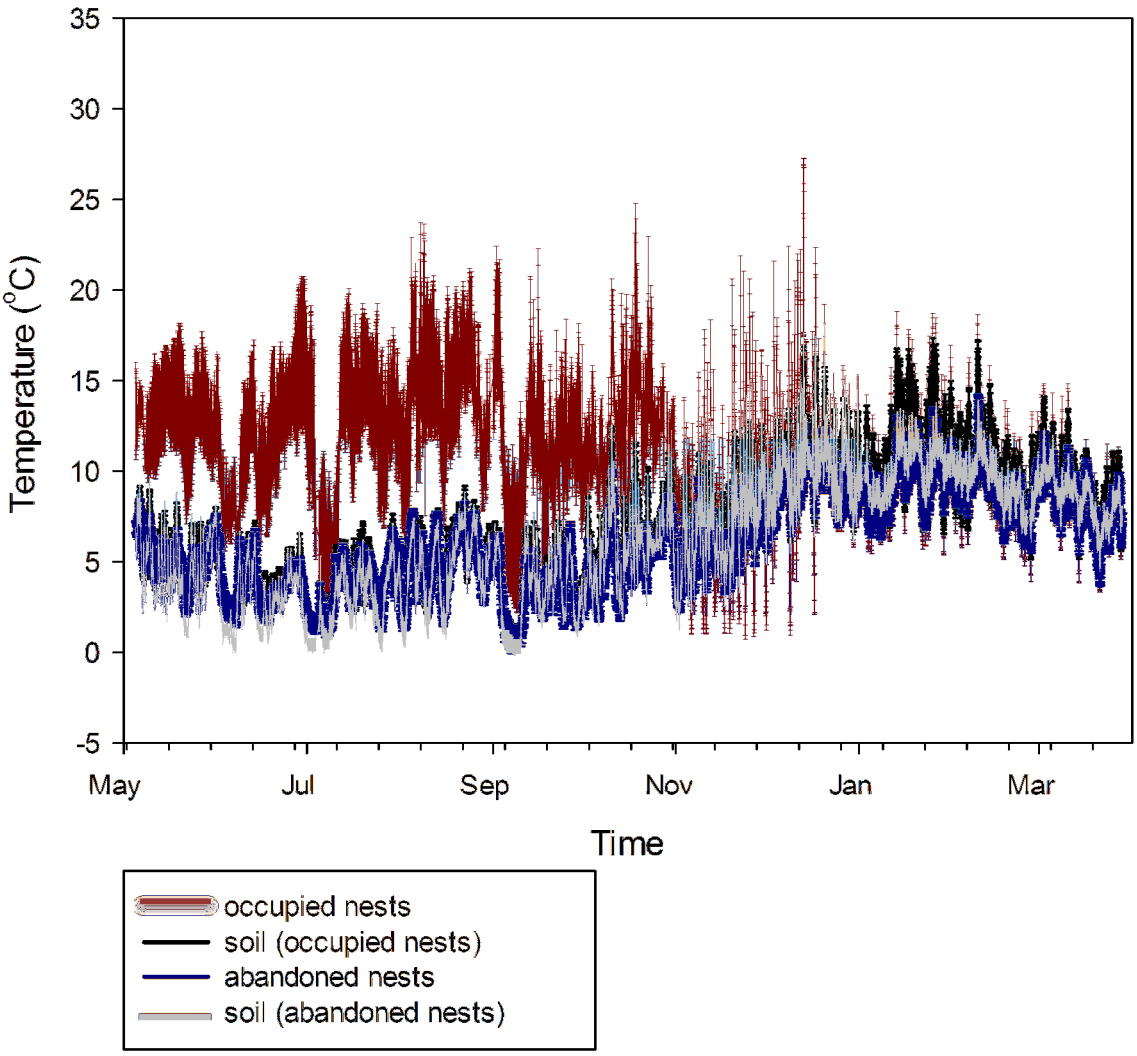

Fig. 3. Mean (± SE) of temperature recorded in occupied and abandoned wandering albatross (*Diomedea exulans*) nests as well as adjacent soil habitats from May 2011 to March 2012.

Table 5. Median and range are given for mean, daily minimum, daily maximum, absolute minimum and absolute maximum temperatures for each month between May 2011 and March 2012 for occupied nests (ON), soil adjacent to occupied nests (OS), abandoned nests (AN) and soil adjacent to abandoned nests (AS).

Month		Mean		Daily minimum		Daily maximum		Absolute minimum		Absolute maximum	
		Median	Range	Median	Range	Median	Range	Median	Range	Median	Range
May' 11	ON	12.1	7.5	11.4	6.5	12.9	8.3	8.6	6.0	15.2	8.5
	OS	5.4	1.0	4.5	7.3	6.7	1.8	2.1	2.0	2.1	2.0
	AN	5.3	1.7	4.7	1.6	6.1	1.7	2.6	2.5	8.1	2.5
	AS	4.8	1.7	3.2	1.4	6.6	2.6	1.0	1.5	9.5	3.4
June' 11	ON	12.3	8.1	11.3	6.7	13.5	9.2	5.1	4.0	18.7	12.5
	OS	3.7	0.8	3.1	1.2	4.7	0.8	1.6	0.6	7.4	1.5
	AN	3.7	1.6	3.3	1.5	4.3	1.8	1.6	1.5	7.6	2.6
	AS	3.1	1.7	2.2	1.8	4.1	2.1	0.5	2.0	6.5	2.0
July' 11	ON	13.5	7.0	12.2	5.4	15.1	8.2	3.6	7.5	20.7	13.5
	OS	3.8	0.6	2.9	0.9	4.7	0.9	1.1	1.0	7.4	3.0
	AN	3.7	1.6	3.1	1.5	4.3	2.0	1.1	1.5	6.6	3.1
	AS	3.0	2.6	2.4	2.8	4.1	2.5	0.5	3.0	7.5	3.0
Aug.'11	ON	15.9	8.8	12.7	6.7	19.1	10.1	4.1	10.6	22.2	15.1
	OS	5.5	0.8	4.1	0.8	6.4	1.9	1.6	2.0	8.6	3.0
	AN	5.0	1.4	4.4	1.7	6.1	2.0	1.6	2.5	8.1	3.6
	AS	4.6	2.2	3.6	2.6	5.8	1.8	1.5	3.5	9.0	2.0
Sep.'11	ON	11.2	5.9	8.9	2.1	13.6	9.4	0.1	3.5	21.7	15.1
	OS	3.8	0.9	2.6	1.0	4.8	1.3	0.6	1.0	7.4	3.0
	AN	3.4	1.6	2.9	1.9	4.2	1.7	0.6	2.1	7.1	3.1
	AS	2.9	1.1	1.9	1.2	4.4	1.3	0.0	2.0	8.0	1.5
Oct.'11	ON	12.9	3.2	9.4	0.7	17.1	6.0	5.1	5.0	23.7	13.5
	OS	5.9	1.2	4.3	0.9	7.6	2.9	1.8	0.6	11.9	6.0
	AN	5.2	1.1	4.2	1.2	6.2	1.8	1.6	2.0	10.1	3.0
	AS	5.7	1.2	3.7	1.2	8.2	1.1	1.5	1.0	13.0	4.5
Nov.'11	ON	7.6	4.0	3.8	8.9	12.4	4.2	1.6	8.5	15.7	3.0
	OS	6.9	1.3	5.3	1.2	9.0	3.0	2.6	2.0	12.7	4.5
	AN	6.2	0.8	5.1	2.1	7.1	1.7	2.6	2.5	9.2	3.0
	AS	7.1	1.5	4.0	1.7	10.5	4.9	1.0	2.0	14.5	7.0
Dec.'11	ON	10.8	2.0	6.6	4.9	15.1	4.2	3.1	9.0	21.7	15.0
	OS	10.2	1.4	8.3	1.0	12.2	3.1	5.6	1.1	16.7	6.0
	AN	9.2	1.1	8.2	0.9	10.2	1.5	5.6	2.0	12.2	2.5
	AS	10.3	1.5	8.1	1.3	13.1	6.2	4.5	2.0	18.5	4.5
Jan.'12	ON	10.7	0.2	9.6	3.3	12.1	4.1	7.1	4.0	15.7	6.0
	OS	10.5	1.4	8.5	1.4	13.1	3.5	6.6	1.1	17.7	6.0
	AN	9.6	1.2	8.5	1.1	10.7	1.7	6.1	1.5	13.7	2.5

table 5 continued

	AS	10.4	1.9	9.6	1.8	11.4	2.4	7.5	1.0	13.5	3.5
Feb.'12	ON	9.9	0.5	9.0	3.0	11.2	3.2	6.1	3.5	15.2	6.0
	OS	9.7	1.2	8.0	1.8	12.0	2.7	5.3	2.1	17.4	6.0
	AN	9.2	1.1	8.2	1.1	10.1	1.4	5.6	1.5	13.7	3.0
	AS	9.2	2.0	8.2	1.7	10.3	2.4	6.5	3.0	13.0	3.5
Mar.'12	ON	9.0	0.5	8.1	2.1	10.1	1.9	4.6	2.0	13.2	3.5
	OS	8.8	0.8	7.6	1.2	10.4	1.8	4.1	2.1	13.9	3.5
	AN	8.4	1.1	7.6	1.3	9.1	1.2	4.1	2.0	11.6	2.0
	AS	8.3	1.5	7.6	1.5	9.4	1.9	5.5	4.5	11.5	5.0

Table 6. Comparing monthly temperature parameters in occupied nests (ON) with adjacent soils (OS), abandoned nests (AN) and soils adjacent to abandoned nests (AS). An example of the comparison is given for the mean temperatures in May. This scheme is followed throughout the table and full results of the GLZ's are available in Table S1.

The first cell will indicate whether the temperature in occupied nests (ON) differs significantly with one or more of the other habitats.
If red = significant difference
If blue = no significant difference

The next three cells compares occupied nests (ON) with each of the other habitats (occupied soil (OS), abandoned nest (AN), or abandoned soil (AS)).
If red and +, significantly higher in ON
If red and -, significantly lower in ON
If blue, no significant difference

	Mean		Daily minimum		Daily maximum		Absolute minimum		Absolute maximum	
May '11	ON with AN (+)	ON with OS (+)	+	+	+	+	+	+	+	+
		ON with AS (+)	+	+	+	+	+	+	+	+
June '11	+	+	+	+	+	+	+	+	+	+
July '11	+	+	+	+	+	+	+	+	+	+
Aug. '11	+	+	+	+	+	+	+	+	+	+
Sep. '11	+	+	+	+	+	+			+	+
Oct. '11	+	+	+	+	+	+	+	+	+	+
Nov. '11	+	+			+	+			+	+
Dec. '11	+	+			+	+			+	+
Jan. '12	+				+	+		-	+	+
Feb. '12					+	+			+	+
March '12	+				+	+				+

Threshold temperatures at which CTmin, freezing and death occurs in *P. marioni* were also recorded. In occupied nests, CTmin threshold events (temperatures < 0.2°C) occurred 50 times compared to 420 in abandoned nests and 842 in soil habitats. The temperatures in nests or soils never reached the thresholds at which freezing (< -3.3°C), or death (lower lethal temperature of < -8.9°C) occurs.

Discussion

By conducting a more extensive sampling effort of caterpillar abundance in three nest types, i.e. old, fledged and new, as well as temperature data recorded over the entire year of nest occupancy, the primary aim of this study was to provide a better understanding of the extent to which wandering albatross serve as thermal ecosystem engineers, and also some of the possible mechanisms which may account for the way this engineering takes place.

The most apparent result by far was that caterpillar abundance was significantly higher in new nests compared to old and fledged nests. Similarly, although caterpillar abundance was not always significantly higher in new nests compared to other vegetation complexes, the coefficient of variation in new nests was substantially lower compared to all other habitats (e.g. Sinclair & Chown 2006). High caterpillar numbers in new nests with low variability, compared to equal or higher numbers in other habitats with high variability, suggest that caterpillars are distributed more evenly within new nests than any other habitat on Marion Island. High densities of *P. marioni* caterpillars have previously been found in *Sanionia uncinata* vegetation communities (Burger 1978). Moreover, *Cotula plumosa* are high nutrient vegetation communities, which could account for the high caterpillar biomass in this habitat type (Smith *et al.* 2001; Smith & Steenkamp 2001), a result consistent with the findings of Sinclair & Chown (2006).

New nests are nests that were built for the current breeding season. Since albatrosses build their nests from tufts of vegetation and peat collected from their surroundings (Warham 1997; Ryan & Bester 2008), one hypothesis that may explain the significantly higher abundance of caterpillars in new compared to older nests, is that eggs or caterpillars may be incidentally incorporated into nests during nest building. Incidental incorporation may be a potential mechanism by which caterpillars come to be in the nests in the first place. However, this hypothesis does not preclude further benefits via either thermal ecosystem engineering or

nutrient input. For example, year-long temperature data revealed that temperatures are indeed significantly higher during nest occupancy compared to abandoned nests and other habitats on the island. Avoidance of low temperature exposure may likely have strong fitness advantages for *P. marioni* caterpillars (Sinclair & Chown 2006). Indeed, in occupied nests, daily minimum and absolute minimum temperatures were significantly higher compared to other habitats, and the number of temperature events at which *P. marioni* experiences chill coma (i.e. CTmin of < 0.2°C) (Klok & Chown 1997) was reduced. Moreover, during the winter months (May to August), temperatures within occupied nests were up to 9°C higher compared to abandoned nests or soils. These estimates are even higher than was previously found for occupied nests (i.e. an increase of 5°C compared to other habitats) (Sinclair & Chown 2006).

Thus, once caterpillars or eggs are added to the nests, favourable nest conditions may allow for continued growth and survival (Sinclair & Chown 2006). Whilst nests were fully occupied (i.e. between May 2011 and November 2012), mean temperatures were anywhere between 8 and 16°C, with a large range of daily minimum (4–13°C) and maximum temperatures (13-19°C). Given that larval development at a fluctuating temperature of 5-15°C is 37 weeks (Haupt *et al.* 2013), and nest temperatures are slightly higher, estimated development time within occupied nests may be even lower (*c.* 30 weeks). On the contrary, mean temperatures in abandoned nests and surrounding soils were between 3 and 10°C during the monitoring period, and the range of minimum and maximum temperatures were small compared to that found in occupied nests. Larval development times are 66 and 46 weeks at 5 and 10°C respectively (Haupt *et al.* 2013), and caterpillars inhabiting soils and abandoned nests may very well develop at similar rates, therefore much longer than that found in occupied nests. In warm nest conditions, caterpillars may be able to complete development, after which they pupate and leave the nests as moths. Given that the overall life cycle of *P. marioni* was found to be quite short, *c.* one year (Haupt *et al*. 2013), that caterpillars vacate the nests once they complete development may explain the significantly lower biomass found in fledged nests. Thus, a link between the year-long immature period of *P. marioni* and the almost year-long nesting and chick rearing cycle of the wandering albatross may be evident (Ryan & Bester 2008; Haupt *et al.* 2013).

Alternatively, another explanation that may account for the high caterpillar abundance in nests is that larvae or ovipositing female moths may seek out nests (Sinclair & Chown 2006). Although caterpillars are unlikely to use chemosensory or thermal cues to search for

nests (Chapter 6), whether female ovipositing moths preferentially seek out nests as a suitable habitat in which to lay their eggs is still not known (Thompson 1988; Nylin & Janz 1993; Huk & Kühne 1999; Scheirs *et al*. 2000; Ganehiarachchi *et al*. 2013). If moths had oviposited their eggs relatively early in the nesting season, then these caterpillars might have already pupated and left the nests thus accounting for the significantly low caterpillar abundance in fledged nests compared to new nests.

In this study, by conducting a more extensive sampling effort of caterpillar abundance in nests, I've shown that new nests do indeed have a significantly higher caterpillar biomass compared to older nests, and that the variation in caterpillar biomass in nests is far lower than that found in other habitats on Marion Island. This result is consistent with the findings by Sinclair & Chown (2006). Moreover, temperature data recorded over a full year showed that temperatures in occupied nests are consistently higher during the entire duration of nest occupancy compared to other habitats on the island.

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Supplementary material

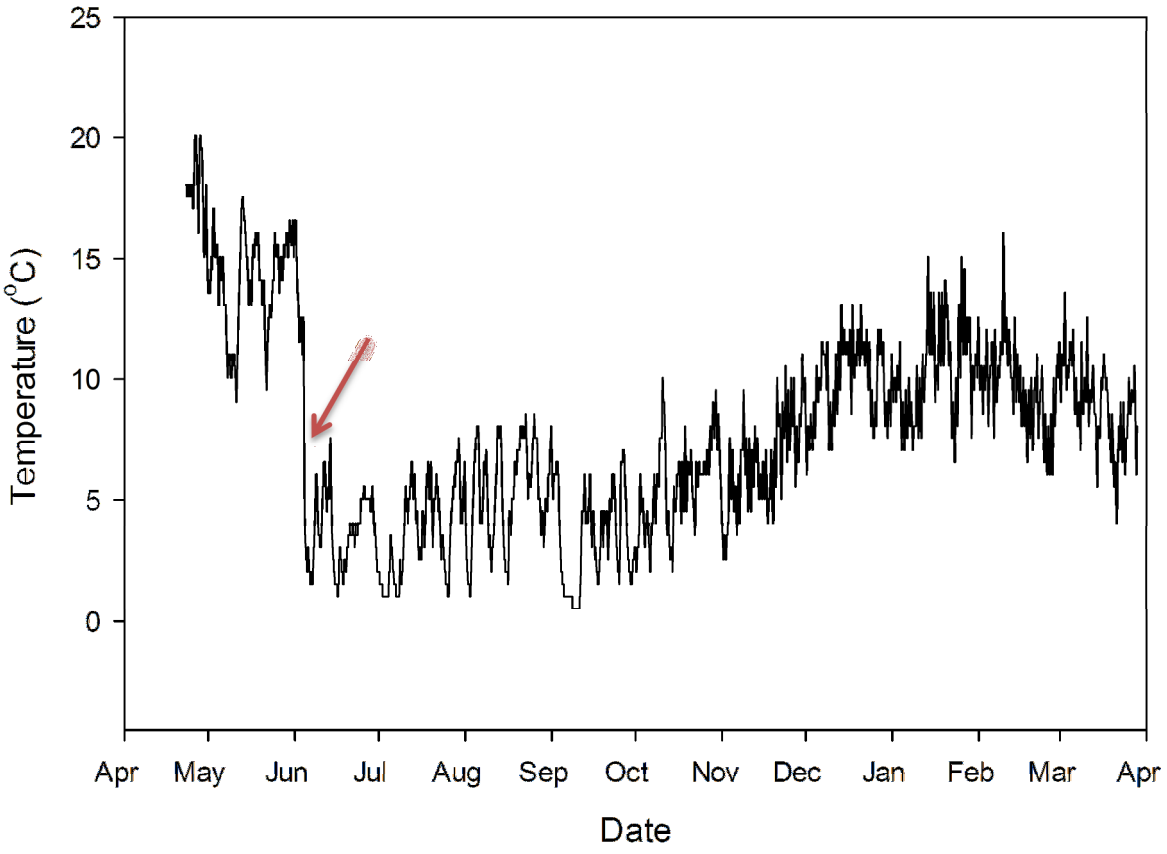

Fig. S1. An example of an occupied nest where the chick had died in June soon after iButtons were deployed. A substantial drop in temperature was observed at this time (indicated by the red arrow).

Table S1. Outputs of the Generalized linear models (Gaussian distribution of errors, log-link for absolute minimum and maximum only) comparing monthly temperature parameters (i.e. mean, daily minimum and maximum, and absolute minimum and maximum temperatures from May 2011 to March 2012) of occupied nests with that of adjacent soils (OS), abandoned nests (AN) and soils adjacent to abandoned nests (AS).

May '11

		Estimate	s.e.	*t*	*P*
Mean	intercept	13.080	0.811	16.137	<0.0001
	type (AN)	-7.804	1.025	-7.612	<0.0001
	type (AS)	-8.497	1.025	-8.287	<0.0001
	type (OS)	-7.669	0.951	-8.068	<0.0001
Daily minimum	intercept	12.222	1.129	10.824	<0.0001
	type (AN)	-7.586	1.428	-5.311	<0.0001
	type (AS)	-9.069	1.428	-6.350	<0.0001
	type (OS)	-7.016	1.324	-5.299	<0.0001
Daily maximum	intercept	13.956	0.931	14.985	<0.0001
	type (AN)	-8.021	1.178	-6.809	<0.0001
	type (AS)	-7.578	1.178	-6.433	<0.0001
	type (OS)	-7.340	1.092	-6.721	<0.0001
Absolute minimum	intercept	5.640	0.003	2147.634	<0.0001
	type (AN)	-0.022	0.003	-6.642	<0.0001
	type (AS)	-0.027	0.003	-7.916	<0.0001
	type (OS)	-0.022	0.003	-7.034	<0.0001
Absolute maximum	intercept	2.792	0.062	44.741	<0.0001
	type (AN)	-0.672	0.113	-5.929	<0.0001
	type (AS)	-0.538	0.104	-5.192	<0.0001
	type (OS)	-0.554	0.091	-6.073	<0.0001

s.e. = standard error

table S1 cont.

June '11

		Estimate	s.e.	*t*	*P*
Mean	intercept	11.702	0.857	13.648	<0.0001
	type (AN)	-8.079	1.085	-7.449	<0.0001
	type (AS)	-8.864	1.085	-8.173	<0.0001
	type (OS)	-7.897	1.005	-7.855	<0.0001
Daily minimum	intercept	10.491	0.747	14.050	<0.0001
	type (AN)	-7.378	0.944	-7.812	<0.0001
	type (AS)	-8.499	0.944	-8.999	<0.0001
	type (OS)	-7.353	0.876	-8.398	<0.0001
Daily maximum	intercept	12.838	0.971	13.223	<0.0001
	type (AN)	-8.626	1.228	-7.024	<0.0001
	type (AS)	-8.974	1.228	-7.308	<0.0001
	type (OS)	-8.209	1.138	-7.210	<0.0001
Absolute minimum	intercept	5.631	0.002	3013.779	<0.0001
	type (AN)	-0.016	0.002	-6.666	<0.0001
	type (AS)	-0.020	0.002	-8.310	<0.0001
	type (OS)	-0.016	0.002	-7.073	<0.0001
Absolute maximum	intercept	2.862	0.076	37.778	<0.0001
	type (AN)	-0.898	0.163	-5.518	<0.0001
	type (AS)	-0.956	0.170	-5.611	<0.0001
	type (OS)	-0.881	0.135	-6.518	<0.0001

s.e. = standard error

table S1 cont.

July '11

		Estimate	s.e.	*t*	*P*
Mean	intercept	11.857	0.817	14.512	<0.0001
	type (AN)	-8.348	1.034	-8.077	<0.0001
	type (AS)	-8.967	1.034	-8.676	<0.0001
	type (OS)	-8.062	0.958	-8.415	<0.0001
Daily minimum	intercept	10.490	0.715	14.677	<0.0001
	type (AN)	-7.609	0.904	-8.416	<0.0001
	type (AS)	-8.442	0.904	-9.338	<0.0001
	type (OS)	-7.508	0.838	-8.958	<0.0001
Daily maximum	intercept	13.288	0.940	14.137	<0.0001
	type (AN)	-9.104	1.189	-7.657	<0.0001
	type (AS)	-9.510	1.189	-7.999	<0.0001
	type (OS)	-8.632	1.102	-7.832	<0.0001
Absolute minimum	intercept	5.624	0.003	1833.989	<0.0001
	type (AN)	-0.011	0.004	-2.776	0.0129
	type (AS)	-0.013	0.004	-3.407	0.0034
	type (OS)	-0.010	0.004	-2.754	0.0136
Absolute maximum	intercept	2.917	0.082	35.525	<0.0001
	type (AN)	-0.997	0.191	-5.221	<0.0001
	type (AS)	-0.916	0.179	-5.119	<0.0001
	type (OS)	-0.886	0.147	-6.025	<0.0001

s.e. = standard error

table S1 cont.

August '11

		Estimate	s.e.	*t*	*P*
Mean	intercept	14.202	0.959	14.811	<0.0001
	type (AN)	-9.208	1.213	-7.591	<0.0001
	type (AS)	-9.578	1.213	-7.896	<0.0001
	type (OS)	-8.876	1.124	-7.893	<0.0001
Daily minimum	intercept	11.952	0.761	15.709	<0.0001
	type (AN)	-7.824	0.962	-8.130	<0.0001
	type (AS)	-8.492	0.962	-8.824	<0.0001
	type (OS)	-7.737	0.892	-8.672	<0.0001
Daily maximum	intercept	16.236	1.179	13.775	<0.0001
	type (AN)	-10.329	1.491	-6.929	<0.0001
	type (AS)	-10.446	1.491	-7.007	<0.0001
	type (OS)	-9.801	1.382	-7.092	<0.0001
Absolute minimum	intercept	5.628	0.004	1322.555	<0.0001
	type (AN)	-0.013	0.005	-2.438	0.0260
	type (AS)	-0.015	0.005	-2.704	0.0151
	type (OS)	-0.012	0.005	-2.459	0.0250
Absolute maximum	intercept	3.036	0.078	39.139	<0.0001
	type (AN)	-0.953	0.174	-5.474	<0.0001
	type (AS)	-0.873	0.163	-5.341	<0.0001
	type (OS)	-0.853	0.136	-6.279	<0.0001

s.e. = standard error

table S1 cont.

September '11

		Estimate	s.e.	*t*	*P*
Mean	intercept	10.816	0.645	16.778	<0.0001
	type (AN)	-7.709	0.815	-9.454	<0.0001
	type (AS)	-7.582	0.815	-9.298	<0.0001
	type (OS)	-7.108	0.756	-9.403	<0.0001
Daily minimum	intercept	8.275	0.388	21.330	<0.0001
	type (AN)	-5.897	0.491	-12.020	<0.0001
	type (AS)	-6.245	0.491	-12.730	<0.0001
	type (OS)	-5.529	0.455	-12.150	<0.0001
Daily maximum	intercept	13.266	0.975	13.608	<0.0001
	type (AN)	-9.302	1.233	-7.544	<0.0001
	type (AS)	-8.739	1.233	-7.087	<0.0001
	type (OS)	-8.555	1.143	-7.484	<0.0001
Absolute minimum	intercept	5.615	0.002	2785.229	<0.0001
	type (AN)	-0.004	0.003	-1.521	0.1470
	type (AS)	-0.004	0.003	-1.649	0.1180
	type (OS)	-0.002	0.002	-0.725	0.4780
Absolute maximum	intercept	3.012	0.078	38.427	<0.0001
	type (AN)	-1.077	0.195	-5.531	<0.0001
	type (AS)	-0.971	0.178	-5.441	<0.0001
	type (OS)	-0.989	0.151	-6.550	<0.0001

s.e. = standard error

table S1 cont.

October '11

		Estimate	s.e.	*t*	*P*
Mean	intercept	12.318	0.408	30.170	<0.0001
	type (AN)	-7.257	0.516	-14.050	<0.0001
	type (AS)	-6.646	0.516	-12.870	<0.0001
	type (OS)	-6.400	0.479	-13.370	<0.0001
Daily minimum	intercept	9.285	0.259	35.860	<0.0001
	type (AN)	-5.154	0.328	-15.730	<0.0001
	type (AS)	-5.442	0.328	-16.610	<0.0001
	type (OS)	-4.857	0.304	-15.990	<0.0001
Daily maximum	intercept	15.256	0.802	19.026	<0.0001
	type (AN)	-9.122	1.014	-8.994	<0.0001
	type (AS)	-7.292	1.014	-7.190	<0.0001
	type (OS)	-7.507	0.940	-7.984	<0.0001
Absolute minimum	intercept	5.625	0.002	2616.849	<0.0001
	type (AN)	-0.010	0.003	-3.594	0.0022
	type (AS)	-0.010	0.003	-3.590	0.0023
	type (OS)	-0.008	0.003	-3.228	0.0049
Absolute maximum	intercept	3.098	0.077	40.458	<0.0001
	type (AN)	-0.843	0.158	-5.347	<0.0001
	type (AS)	-0.481	0.123	-3.917	0.0011
	type (OS)	-0.623	0.116	-5.359	<0.0001

s.e. = standard error

table S1 cont.

November '11

		Estimate	s.e.	*t*	*P*
Mean	intercept	8.763	0.521	16.834	<0.0001
	type (AN)	-2.764	0.658	-4.198	0.0006
	type (AS)	-1.856	0.658	-2.818	0.0119
	type (OS)	-1.792	0.610	-2.936	0.0092
Daily minimum	intercept	5.401	0.982	5.502	<0.0001
	type (AN)	-0.492	1.242	-0.396	0.6970
	type (AS)	-1.214	1.242	-0.978	0.3420
	type (OS)	-0.184	1.151	-0.160	0.8750
Daily maximum	intercept	13.635	0.830	16.431	<0.0001
	type (AN)	-6.385	1.050	-6.083	<0.0001
	type (AS)	-2.821	1.050	-2.688	0.0156
	type (OS)	-4.391	0.973	-4.513	0.0003
Absolute minimum	intercept	5.619	0.003	1641.753	<0.0001
	type (AN)	-0.0001	0.004	-0.018	0.9860
	type (AS)	-0.005	0.004	-1.110	0.2820
	type (OS)	0.001	0.004	0.164	0.8720
Absolute maximum	intercept	2.792	0.066	42.253	<0.0001
	type (AN)	-0.506	0.108	-4.705	0.0002
	type (AS)	-0.071	0.086	-0.828	0.4193
	type (OS)	-0.231	0.083	-2.770	0.0131

s.e. = standard error

table S1 cont.

December '11

		Estimate	s.e.	*t*	*P*
Mean	intercept	11.055	0.333	33.187	<0.0001
	type (AN)	-1.844	0.421	-4.377	0.0004
	type (AS)	-0.971	0.421	-2.305	0.0341
	type (OS)	-0.934	0.391	-2.392	0.0286
Daily minimum	intercept	8.214	0.608	13.513	<0.0001
	type (AN)	0.062	0.769	0.080	0.9370
	type (AS)	-0.369	0.769	-0.480	0.6370
	type (OS)	0.158	0.713	0.222	0.8270
Daily maximum	intercept	15.031	0.946	15.893	<0.0001
	type (AN)	-4.809	1.196	-4.020	0.0009
	type (AS)	-3.150	1.196	-2.633	0.0174
	type (OS)	-2.651	1.109	-2.391	0.0287
Absolute minimum	intercept	5.622	0.003	1620.944	<0.0001
	type (AN)	0.008	0.004	1.885	0.0766
	type (AS)	0.004	0.004	0.964	0.3485
	type (OS)	0.008	0.004	1.941	0.0690
Absolute maximum	intercept	3.135	0.079	39.657	<0.0001
	type (AN)	-0.614	0.138	-4.447	0.0004
	type (AS)	-0.212	0.109	-1.937	0.0695
	type (OS)	-0.327	0.104	-3.150	0.0058

s.e. = standard error

table S1 cont.

January '12

		Estimate	s.e.	*t*	*P*
Mean	intercept	10.632	0.338	31.468	<0.0001
	type (AN)	-1.169	0.427	-2.735	0.0141
	type (AS)	-0.428	0.427	-1.001	0.3310
	type (OS)	-0.130	0.396	-0.328	0.7467
Daily minimum	intercept	8.505	0.485	17.529	<0.0001
	type (AN)	0.011	0.614	0.017	0.9870
	type (AS)	0.859	0.614	1.399	0.1800
	type (OS)	-0.019	0.569	-0.034	0.9730
Daily maximum	intercept	13.395	0.752	17.818	<0.0001
	type (AN)	-2.869	0.951	-3.017	0.0078
	type (AS)	-2.225	0.951	-2.340	0.0318
	type (OS)	-0.272	0.882	-0.309	0.7612
Absolute minimum	intercept	5.632	0.002	3056.056	<0.0001
	type (AN)	0.0004	0.002	0.163	0.8727
	type (AS)	0.005	0.002	2.279	0.0359
	type (OS)	0.001	0.002	0.318	0.7547
Absolute maximum	intercept	2.852	0.063	45.167	<0.0001
	type (AN)	-0.276	0.090	-3.056	0.0072
	type (AS)	-0.235	0.088	-2.655	0.0167
	type (OS)	-0.003	0.074	-0.039	0.9695

s.e. = standard error

table S1 cont.

February '12

		Estimate	s.e.	*t*	*P*
Mean	intercept	9.792	0.331	29.618	<0.0001
	type (AN)	-0.795	0.418	-1.901	0.0743
	type (AS)	-0.329	0.418	-0.787	0.442
	type (OS)	-0.105	0.3877	-0.271	0.7897
Daily minimum	intercept	8.006	0.475	16.858	<0.0001
	type (AN)	0.157	0.601	0.261	0.7970
	type (AS)	0.691	0.601	1.150	0.2660
	type (OS)	-0.046	0.557	-0.082	0.9360
Daily maximum	intercept	12.171	0.611	19.920	<0.0001
	type (AN)	-2.241	0.773	-2.899	0.0100
	type (AS)	-1.853	0.773	-2.398	0.0282
	type (OS)	-0.327	0.717	-0.456	0.6540
Absolute minimum	intercept	5.628	0.002	2614.939	<0.0001
	type (AN)	0.002	0.003	0.892	0.3848
	type (AS)	0.005	0.003	1.923	0.0714
	type (OS)	0.001	0.003	0.334	0.7425
Absolute maximum	intercept	2.842	0.069	41.310	<0.0001
	type (AN)	-0.221	0.096	-2.312	0.0335
	type (AS)	-0.247	0.097	-2.550	0.0207
	type (OS)	-0.008	0.081	-0.096	0.9247

s.e. = standard error

table S1 cont.

March '12

		Estimate	s.e.	*t*	*P*
Mean	intercept	8.875	0.252	35.189	<0.0001
	type (AN)	-0.675	0.319	-2.115	0.0495
	type (AS)	-0.369	0.319	-1.157	0.2633
	type (OS)	-0.059	0.296	-0.200	0.8438
Daily minimum	intercept	7.485	0.374	20.014	<0.0001
	type (AN)	-0.091	0.473	-0.192	0.8500
	type (AS)	0.382	0.473	0.808	0.4300
	type (OS)	0.049	0.439	0.111	0.9130
Daily maximum	intercept	10.642	0.415	25.619	<0.0001
	type (AN)	-1.493	0.525	-2.842	0.0113
	type (AS)	-1.478	0.525	-2.812	0.0120
	type (OS)	-0.259	0.487	-0.532	0.6014
Absolute minimum	intercept	0.0003	0.002	2443.500	<0.0001
	type (AN)	0.000	0.003	0.090	0.9290
	type (AS)	0.004	0.003	1.440	0.1680
	type (OS)	0.001	0.003	0.230	0.8200
Absolute maximum	intercept	2.661	0.062	42.588	<0.0001
	type (AN)	-0.189	0.086	-2.213	0.0509
	type (AS)	-0.185	0.085	-2.163	0.0450
	type (OS)	-0.026	0.074	-0.346	0.7337

s.e. = standard error

Chapter 4 – The effects of temperature on the metabolic response of a keystone sub-Antarctic insect species[1]

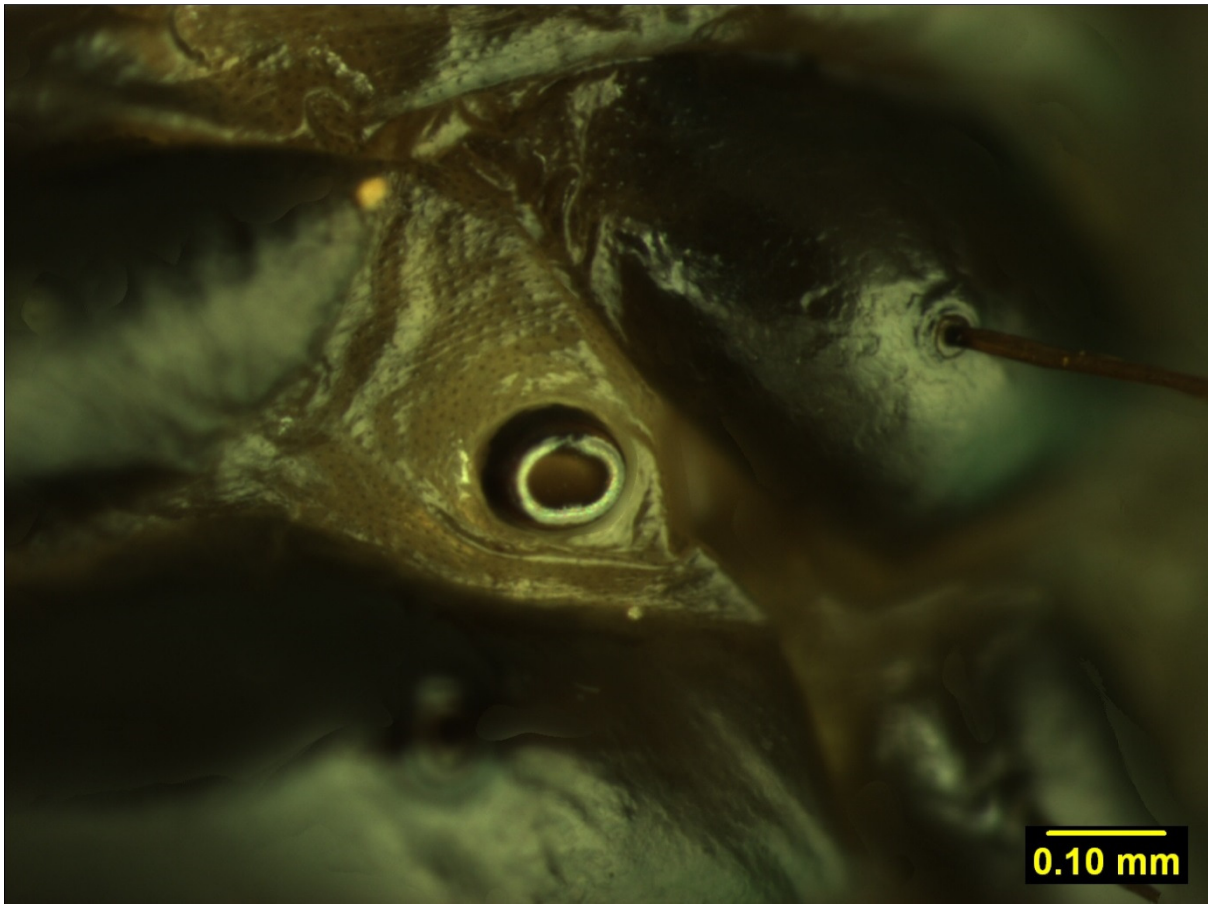

Image of a spiracle of a *Pringleophaga marioni* caterpillar.
Photo Credit: C. Janion-Scheepers

[1]To be submitted as: T.M. Haupt, B.J. Sinclair & S.L. Chown. The effects of temperature on the metabolic response of a keystone sub-Antarctic species. *The Journal of Experimental Biology*

Abstract

Metabolic rate is a fundamental measure of physiological activity, with the primary function being to fuel all biological processes. Thus, knowing how organisms may respond metabolically to changing temperature regimes is important. Variation in metabolic rate in response to environmental conditions has been explained in the context of two contrasting hypotheses: (i) the elevation of metabolism at low temperatures which may allow development to be completed in extreme conditions (i.e. metabolic cold adaption (MCA)), and (ii) metabolic depression in response to stressful or resource limited conditions (e.g. high temperature extremes in variable environments and water loss). In this study, a strong inference approach is used to examine how temperature (both constant and fluctuating), as well as water loss, may affect the metabolic response of caterpillars of the flightless moth, *Pringleophaga marioni*, on sub-Antarctic Marion Island. Partial support for metabolic cold adaptation is shown, and caterpillars are unlikely to depress their metabolic rates to conserve water. On Marion Island, temperatures are persistently cold and habitats of this species are often waterlogged. It is therefore likely that selection may favour adaptive strategies which allow development to be completed in cold conditions, rather than conserving water. In addition, caterpillars are unlikely to decrease their thermal sensitivity in response to fluctuating temperatures. However, significant metabolic depression after exposure to a fluctuating temperature of 5-15°C suggests additional metabolic costs at this variable temperature regime. This latter result may likely be a consequence of stress after exposure to the high temperature extreme, a result consistent with the finding of high temperatures being detrimental to the survival of *P. marioni* larvae.

Keywords: sub-Antarctic - metabolic cold adaption - metabolic rate - water loss - Jensen's inequality

Introduction

Because ectotherms are typically unable to use metabolically produced heat to regulate their body temperatures, they have developed strategies to cope with environmental temperature variation. Among the most controversial is metabolic cold adaptation (Chown & Gaston 1999; Addo-Bediako *et al.* 2002; Clarke 2003). In general terms, to compensate for exposure to cold conditions, animals may elevate or maintain their metabolic rates. This may be evident either via a higher metabolic rate at a common test temperature compared to exposure to warmer conditions, or, reduced sensitivity (i.e. lower slope) of the metabolic rate temperature (MR-T) curve (Addo-Bediako *et al.* 2002; Irlich *et al.* 2009; Terblanche *et al.* 2009).

An elevated metabolic rate in cold conditions provides a fitness advantage as it allows for higher levels of activity such that growth and development may be completed (Sømme & Block 1991). Among arthropods, metabolic cold adaptation has been found in beetles (e.g. Aunaas *et al.* 1983; Strømme *et al.* 1986; Schultz *et al.* 1992; Chown *et al.* 1997), grasshoppers (Chappell 1983; Massion 1983; Hadley & Massion 1985), mites (Block and Tilbrook 1975; Block 1976; Block & Young 1978) and more recently, vinegar flies (Berrigan & Partridge 1997) and tsetse flies (Terblanche *et al.* 2009).

It is also argued that as maintaining or elevating metabolism are energy intensive processes, this would result in greater metabolic costs (Clarke 1991; 1993; 2003). There is therefore no selective benefit in maintaining or elevating metabolic rate and metabolic cold adaptation is unlikely to evolve (Clarke 1991). Thus, studies on arthropods have also found little support for metabolic cold adaptation in flies (Lee & Baust 1982a; Chown 1997), ticks (Lee & Baust 1982b), beetles (Nylund 1991), caterpillars (Bennett *et al.* 1999), woodlice (Lardies *et al.* 2004), and springtails (McGaughran *et al.* 2010). Instead of an elevated metabolic rate in low temperature conditions, ectotherms may respond to stressful conditions such as high temperatures, food, or water stress, by depressing their metabolism (Clarke 1993).

In insects, water loss occurs during respiration via the spiracles (Hadley 1994), and higher rates of gas exchange would entail higher water loss rates (Kestler 1985; Lighton 1996; Woods & Smith 2010). A lowered metabolic rate at high temperatures or in dry conditions would mean that less water is lost to the environment. Termed the "water conservation" hypothesis (reviews in Kestler 1985; Zachariassen 1996; Chown & Gaston 1999; Addo-Bediako *et al.* 2001; Chown 2002; Chown *et al.* 2011), based on how significant the contribution of respiratory water loss is to total water loss, studies have either supported

this idea (e.g. Lighton & Bartholomew 1988; Chown & Davis 2003; Gibbs *et al.* 2003; Schimpf *et al.* 2009) or have refuted it (e.g. Quinlan & Hadley 1993; Djawdan *et al.* 1997; Williams & Bradley 1998; Williams *et al.* 1998; Bosch *et al.* 2000; Rourke 2000).

In addition, a lowered metabolic rate at high temperatures may be either a strategy to reduce water, or a case of metabolic cold adaptation, as both hypotheses predict a lowered metabolic rate at high temperatures compared to lower temperatures (Davis *et al.* 2000). If water conservation were responsible for the metabolic response, then respiratory water loss would be lowest under dry conditions (Keister & Buck 1964). These predictions are contrary to what is generally expected, i.e. in warmer temperatures, gas exchange occurs more rapidly and higher rates of gas exchange would mean greater water loss rates (Woods & Smith 2010). Terblanche *et al.* (2010) found that in Scarabaeidae dung beetles, both cuticular and respiratory water loss reduction was observed at high temperatures of 25°C (by comparison with 15°C and 20°C), with the latter being associated with a marked depression of metabolic rate. They suggested that the change in metabolic rate was not a form of metabolic cold adaptation, but rather a metabolic response to increasing water loss rates at higher temperatures (Terblanche *et al.* 2010).

Furthermore, fluctuating temperatures are a characteristic feature of the natural environment, and metabolic rate may vary in response to stable vs. variable environments (e.g. Lalouette *et al.* 2011; Niehaus *et al.* 2011; Williams *et al.* 2012). This response follows from Jensen's inequality, which is a mathematical property of non-linear functions such as thermal fitness curves (Ruel & Ayres 1999). Jensen's inequality states that the effects of temperature on ectotherm performance cannot be predicted by stable temperatures alone, but depend strongly on the variance in temperature experienced in any given environment (Ruel & Ayres 1999). Thus, when measuring ectotherm performance or life history traits, physiologists have begun to examine fluctuating temperatures along with stable ones as variable environments represent more ecologically-realistic conditions (e.g. Folguera *et al*. 2008; Ragland & Kingsolver 2008; Folguera *et al*. 2009; Kingsolver *et al.* 2009; Paaijmans *et al*. 2010; Estay *et al*. 2011; Williams *et al.* 2012; Lyons *et al.* 2013). Because of the exponential nature of the MR-T relationship, the accelerating function of the MR-T curve will result in an increase in metabolism at high temperatures (Ruel & Ayres 1999). Thus, in fluctuating environments, metabolic rate will be disproportionately higher during the warm periods than it is lower during the cool periods, leading to an elevation of the overall mean (Ruel & Ayres 1999). A high metabolism may mean greater metabolic costs as more energy is required to maintain it (Ruel & Ayres 1999). Theory predicts that ectotherms may avoid

these associated costs by lowering their thermal sensitivity of metabolism, which will in turn, reduce the impact of the warm portion of thermal variability (Pásztor *et al*. 2000; Niehaus *et al.* 2011; Williams *et al*. 2012).

From the above discussion, it is evident that the metabolic rate of ectotherms may be either, elevated in response to low temperatures, maximised at high temperature extremes, or depressed in response to stressful conditions (Sømme & Block 1991; Ayres & Scriber 1994; Chown & Gaston 1999; Ruel & Ayres 1999; Addo-Bediako *et al.* 2002; Terblanche *et al.* 2009). Thus, when examining the effects of temperature on the metabolic response of organisms, it is important that additional factors such as water loss and the effects of variable environments are also considered (e.g. Clusella-Trullas & Chown 2008; Schimpf *et al.* 2009; Terblanche *et al.* 2010; Williams *et al.* 2012; Weldon *et al.* 2013).

The primary aim of this study is to examine how temperature affects the metabolic response of sub-Antarctic flightless moth caterpillars, *Pringleophaga marioni*. In addition, the extent to which water loss may drive the metabolic response, as well as the effects of fluctuating temperatures are also examined. By acclimating caterpillars to both constant and fluctuating temperature regimes, I investigate the hypotheses of metabolic cold adaptation and water conservation, and test whether caterpillars respond to the effects of Jensen's inequality on metabolic rates by subsequent shifts in the MR-T relationships. On Marion Island where temperatures are persistently low (Le Roux & McGeogh 2008), I predict that caterpillars may elevate their metabolic rates in low temperature conditions to maximise activity such that growth and development may be completed. If so, support for the metabolic cold adaption hypotheses may be expected. Alternatively, caterpillars may also depress their metabolism in high temperature conditions so as to conserve water. If so, I predict that water loss will be significantly reduced in high temperature conditions and the water conservation hypothesis may be supported. Moreover, higher mean metabolic rates at fluctuating temperatures may prove costly, and caterpillars may respond by depressing their metabolic rates under variable temperature regimes.

Materials and methods

Study site and species collection

Marion Island (46°54'S, 37°45'E) forms part of the Prince Edward Island group where *P. marioni* is found in virtually all lowland habitats as well as at higher altitudes (Crafford *et al.* 1986). Caterpillars were collected from abandoned wandering albatross nests in the vicinity of the research station on the last day of the April to May 2011 relief voyage and returned to

the laboratory within six hours after collection. Individuals were placed separately in Petri dishes with albatross nest material, which served as both refuge and food (see Haupt *et al*. 2013), and were transferred under refrigeration at *c.* 5°C to the laboratory in Stellenbosch within six days. Keeping individuals separate was necessary to avoid cannibalism (French & Smith 1983). Caterpillars were kept in temperature chambers at 5°C (MIR 154, Sanyo, Osaka, Japan, accurate to ± 0.5°C) before being acclimated at constant temperatures of 5°C, 10°C, and 15°C, or fluctuating temperatures of 0-10°C, 5-15°C, and 10-20°C. Temperatures are similar to soil microhabitat temperatures on Marion Island (Chown & Crafford 1992; S.L. Chown, unpublished data). The photoperiod was set at 12L:12D. The acclimation time was seven days, after which respirometry trials commenced (n = 18 caterpillars per acclimation treatment). The timing of acclimation period was based on previous trials showing acclimation responses within a week for this species (Sinclair & Chown 2003) and for insects generally (Weldon *et al*. 2011).

Experimental procedure

The rate of CO_2 loss (VCO_2) as an indicator of metabolic rate (MR) and total water loss rate (TWLR) as VH_2O, were measured simultaneously using flow-through respirometry. Trial runs were undertaken on individual caterpillars at test temperatures of 0°C, 5°C, 10°C and 15°C in randomised order, except for 15°C, which was tested last, in the instance of any deleterious effects at this high temperature. At the start and end of each run, individuals were weighed on a balance (FA324T, Avery Berkel, South Africa) and the final mass obtained by calculating the mean of the start and end mass. Three respirometry systems were run concurrently to obtain measurements for several individuals at one time (an example of one set-up is shown in Appendix A). For each set-up, bottled air (21% O_2, balance N) flowed into cylinders containing soda lime, silica gel and drierite, which scrubbed air of residual CO_2 and H_2O. Air flowed at 200 ml min^{-1} and flow rate was controlled using a Side-track (Sierra Instruments, Monteray, CA, USA) mass flow controller. This dry CO_2-free air flowed through narrow Bev-A-Line plastic tubing (Cole-Parmer, USA) into a cuvette in which an individual caterpillar was held. The cuvette was held on an activity detector (Sable Systems, Henderson, NV), which monitored the activity of the caterpillar. The cuvette was covered in foil to eliminate the effects of light. The activity detector plus the cuvette were enclosed inside a Ziploc bag and placed into a Grant LTC 12 water bath (Grant Instruments, Cambridge, UK) set at the desired test temperature. The temperature within the cuvettes were measured with Type T 40 gauge thermocouples and logged using a TC-1000 thermocouple

thermometer (Sable Systems, Henderson, NV, USA), or a digital thermometer (CHY 507, Taiwan). Only when the temperature in the cuvette matched that of the test temperature, did trial runs begin. From the cuvette, excurrent air flowed into a LiCor 7000 CO_2/H_2O analyser (LiCor, Lincoln, NE, USA), where VCO_2 and VH_2O were quantified. All electronic units in the respirometry system were connected to a desktop computer and controlled by DATACAN V software (Sable Systems, Las Vegas, NV, USA). For each run, a baseline measurement was taken before the caterpillar was placed into the cuvette, as well as at the end after the caterpillar had been removed. Trial runs lasted for 60 min, within which VCO_2 and VH_2O were recorded every second.

Data analyses

The data for each individual were imported into Expedata (Sable Systems) and drift corrected to baseline values. CO_2 data were transformed from micromoles per mole (µm m^{-1}) to millilitres per hour (ml h^{-1}) and H_2O from millimoles per mole (mmol m^{-1}) to milligrams per hour (mg h^{-1}) (Lighton 2008). To generate the resting metabolic rate of each caterpillar at each test temperature, a 5-10 min period where VCO_2 production was low and stable was analysed. Corresponding total water loss rates (VH_2O) were calculated using this same period. Measurements of VCO_2 were converted to microwatts (µW) assuming a joule equivalence of 24.65 kJ L^{-1} (Chown *et al.* 2007). To extract respiratory water loss rate from total water loss rate, the intercept method by Gibbs & Johnson (2004), whereby VCO_2 is regressed against VH_2O, was used. Metabolic rate (MR), total water loss rate (TWLR) and body mass distributions were $\log_{10}$ transformed prior to analyses to obtain, or in a few instances, improve the normality of the data.

To test metabolic cold adaptation and water conservation, the effects of constant acclimation temperatures (5°C, 10°C and 15°C) on MR and TWLR were first compared using an Analysis of Covariance (ANCOVA) which tested for differences in slopes and intercepts. This model required that the 15°C test temperature be removed, as only the linear part of rate-temperature relationships are investigated (Irlich *et al.* 2009). This approach was used in all subsequent ANCOVA analyses. To obtain the best-fit model, model simplification by stepwise deletion of non-significant interaction terms were used (Crawley 2007). Using this approach, the final ANCOVA models for both MR and TWLR included acclimation as a categorical variable, test temperature as a continuous variable, and mass as a covariate.

The effects of acclimation and test temperature on MR and TWLR were then further examined using orthogonal polynomial contrast analysis (Huey *et al.* 1999). These analyses

investigated the linear and quadratic effects of acclimation and test temperature to determine whether metabolic cold adaptation was present or whether an alternative acclimation hypothesis was supported (see Huey *et al.* (1999), Deere & Chown (2006) for rationale and approaches). In these analyses, the 15°C test temperature was retained, and test temperature and acclimation were included as ordered factors in the model, with mass as a covariate. For TWLR, model simplification revealed a significant result for the interaction between acclimation and mass and this interaction term was retained in the final model.

An ANCOVA was used to examine the effects of acclimation on MR at each fluctuating acclimation temperature with its comparable mean constant temperature (i.e. 5°C and 0-10°C, 10°C and 5-15°C, 15°C and 10-20°C). For all comparisons, the 15°C test temperature was removed, and the best-fit model via model simplification included test temperature as a continuous variable, mass as a covariate, and acclimation as a categorical variable.

ANCOVA and Orthogonal polynomial contrast analyses require strict adherence to the assumptions of ANOVA, which are normally distributed residuals, homogeneity of variance, and a balanced design (Huey *et al.* 1999). Formal tests on MR and TWLR showed instances of non-normality (Shapiro-Wilk's), but variances were homogenous (Levene's) (Table S1), and upon log transformation of both MR and TWLR, plots of the residuals (i.e. residual vs fitted values, histogram and probability plots) indicated normally distributed residuals with homogenous variances (see supplementary figures). Analyses were implemented in R3.0.0 (R core team, 2013).

Results

Rate temperature relationships were curvilinear in all cases, with very similar forms (Table 1, Fig. 1). Constant acclimation temperatures of 5°C, 10°C and 15°C had no effect on either metabolic rate (MR) or total water loss rate (TWLR) in the initial ANCOVA analyses, whereas both test temperature and mass had a significant effect (Table 2 and 3). In addition, no interaction terms were significant in the maximal model indicating no variation in slopes for both MR and TWLR.

Table 1. Summary statistics showing means and standard errors (m ± SE) of resting metabolic rate (VCO_2 µW), water loss rate (VH_2O mg h^{-1}) and body mass (g) of *Pringleophaga marioni* caterpillars under six acclimation and four test temperatures. Measurements of VCO_2 and VH_2O are comparable to those obtained for the same species in a study by Sinclair *et al.* (2004).

Acc (°C)	TT (°C)	Body mass (g)	VCO_2 (µW)	VH_2O (mg h^{-1})
5	0	0.1300 ± 0.0086	16.2070 ± 2.0575	2.1406 ± 0.3858
5	5	0.1280 ± 0.0074	38.4943 ± 5.7476	3.0797 ± 0.3833
5	10	0.1286 ± 0.0075	61.7232 ± 7.4045	4.71686 ± 0.5768
5	15	0.1135 ± 0.0074	74.2254 ± 7.3246	6.0105 ± 0.7245
10	0	0.1110 ± 0.0084	16.6440 ± 3.5461	1.70865 ± 0.2678
10	5	0.1111 ± 0.0088	34.5443 ± 4.5488	3.1189 ± 0.4834
10	10	0.1115 ± 0.0089	66.5075 ± 8.2582	3.5228 ± 0.5598
10	15	0.10786 ± 0.0148	66.5297 ± 6.9845	3.5563 ± 0.4755
15	0	0.1281 ± 0.0097	10.9998 ± 1.1868	2.3467 ± 0.4492
15	5	0.1272 ± 0.0084	31.8059 ± 4.5229	2.4730 ± 0.4147
15	10	0.1265 ± 0.0091	62.4958 ± 8.8980	4.1613 ± 0.5335
15	15	0.1082 ± 0.0078	67.7387 ± 10.6730	4.1711 ± 0.4977
0~10	0	0.1288 ± 0.0109	12.6696 ± 1.2262	3.1735 ± 0.5539
0~10	5	0.1278 ± 0.0121	35.3060 ± 4.3583	3.8069 ± 0.7428
0~10	10	0.1285 ± 0.0109	60.6870 ± 6.1218	5.3866 ± 0.7817
0~10	15	0.1061 ± 0.0094	61.7979 ± 6.5244	6.0969 ± 0.8363
5~15	0	0.1205 ± 0.0098	9.0485 ± 1.1029	2.0079 ± 0.2557
5~15	5	0.1191 ± 0.0113	25.1861 ± 3.5139	2.7137 ± 0.5209
5~15	10	0.1184 ± 0.0104	44.3947 ± 5.2929	3.5106 ± 0.5441
5~15	15	0.1027 ± 0.0097	58.2723 ± 8.5317	4.2478 ± 0.6205
10~20	0	0.1393 ± 0.0102	13.5168 ± 2.1387	1.9909 ± 0.3681
10~20	5	0.1410 ± 0.0116	31.6486 ± 4.3511	3.2101 ± 0.6818
10~20	10	0.1433 ± 0.0123	58.9488 ± 5.9630	4.5122 ± 0.7511
10~20	15	0.1227 ± 0.0096	61.2179 ± 7.8749	4.2793 ± 0.6128

Acc = acclimation; TT = test temperature

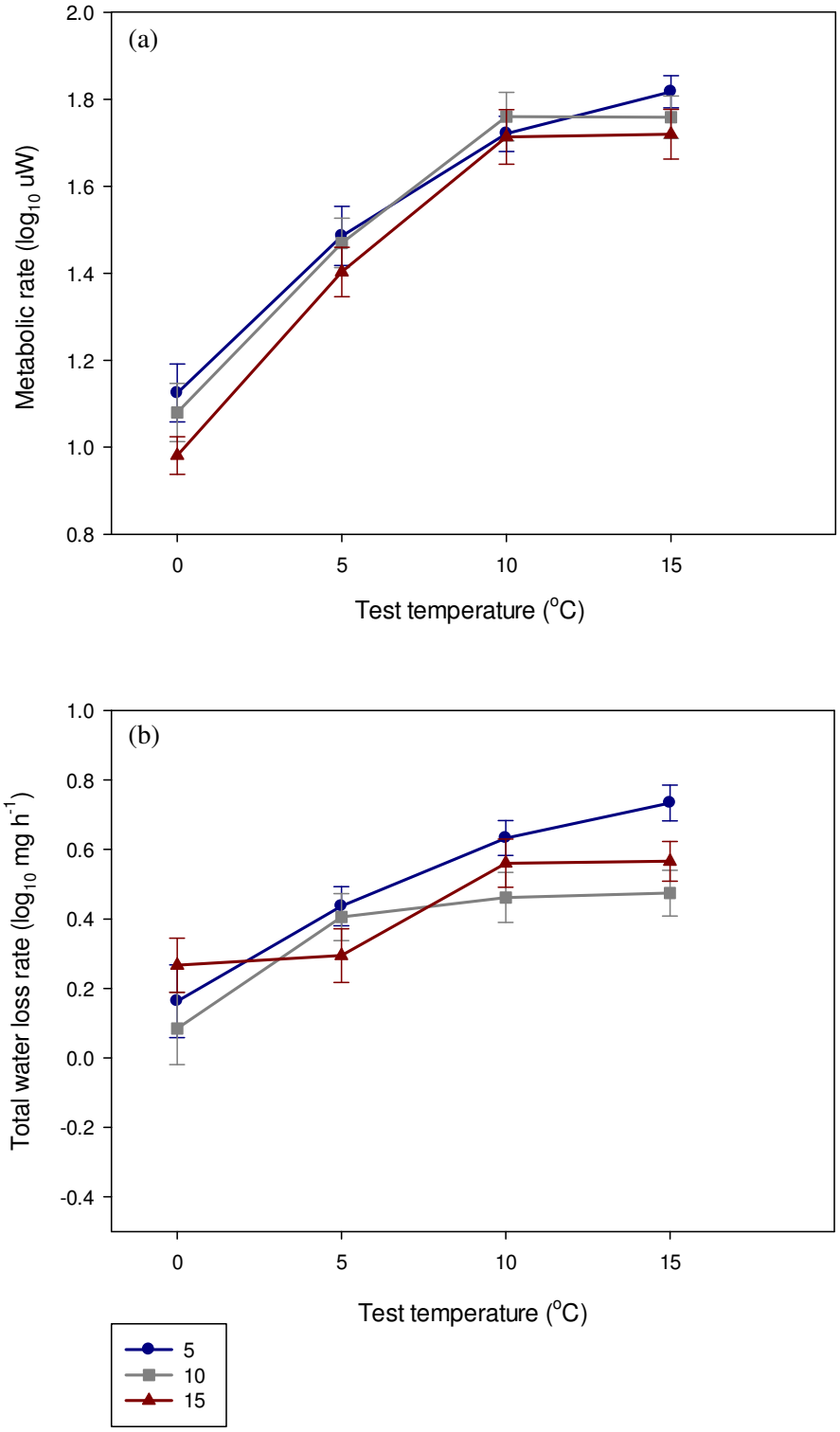

Fig. 1. The metabolic rate (log_{10} µW) (**a**) and total water loss rate (log_{10} mg h^{-1}) (**b**) of *Pringleophaga marioni* caterpillars at test temperatures of 0°C to 15°C after acclimation to constant temperatures of 5°C (blue), 10°C (grey) and 15°C (red). Means ± SE are shown.

Table 2. Results from an analyses of covariance comparing the metabolic rate ($\log_{10}$ μW) of *Pringleophaga marioni* caterpillars between constant acclimation temperatures of 5°C, 10°C and 15°C.

	d.f.	SS	MS	*F*-value	*P*
acc	2	0.200	0.1	2.125	0.123
tt	**1**	**12.115**	**12.115**	**257.003**	**< 0.0001**
m	**1**	**2.142**	**2.142**	**45.443**	**< 0.0001**
		Estimate	s.e.	*t*	*P*
intercept		**1.913**	**0.125**	**15.338**	**< 0.0001**
acc 10		0.052	0.043	1.210	0.228
acc 15		-0.078	0.042	-1.875	0.0626
tt		**0.067**	**0.004**	**16.118**	**< 0.0001**
m		**0.881**	**0.131**	**6.741**	**< 0.0001**

acc = acclimation; tt = test temperature

SS = sums of squares; MS = mean squares; s.e. = standard error

Bold indicates significant effects

Table 3. Results from an analyses of covariance comparing the total water loss rate (log_{10} mg h^{-1}) of *Pringleophaga marioni* caterpillars between constant acclimation temperatures of 5°C, 10°C and 15°C.

	d.f.	SS	MS	*F*-value	*P*
acc	2	0.240	0.120	1.249	0.290
tt	**1**	**3.907**	**3.907**	**40.601**	**< 0.0001**
m	**1**	**2.055**	**2.055**	**21.355**	**< 0.0001**
		Estimate	s.e.	*t*	*P*
intercept		**1.007**	**0.178**	**5.662**	**<0.0001**
acc 10		-0.038	0.061	-0.630	0.5300
acc 15		-0.038	0.060	-0.628	0.5310
tt		**0.038**	**0.006**	**6.398**	**< 0.0001**
m		**0.859**	**0.186**	**4.621**	**< 0.0001**

acc = acclimation; tt = test temperature

SS = sums of squares; MS = mean squares; s.e. = standard error

Bold indicates significant effects

In contrast, acclimation was shown to have a significant effect on both MR and TWLR in the orthogonal polynomial contrast analyses. For metabolic rate, a significant negative linear effect of acclimation was found (Table 4): at the colder acclimation temperature of 5°C, values for MR at all test temperatures were mostly higher than at the warm acclimation temperature of 15°C (Table 1; Fig. 1a). A significant negative quadratic effect of acclimation was also found (Fig. 1a). Furthermore, MR increased from 0°C to 10°C, while the 15°C test temperature appeared to be the inflection point at which MR declined or stabilized (Table 1; Fig. 1a). This was shown by a positive linear effect of test temperature, followed by a negative quadratic effect (Table 4). For TWLR, a significant quadratic effect for acclimation and the interaction between acclimation and mass was found (Table 5). The acclimation effect was largely as a result of differences in TWLR between the 5°C and 10°C acclimation temperatures, with the former acclimation temperature having higher values at the 10°C and 15°C test temperatures (Table 1; Fig. 1b). The significant interaction between acclimation and mass may indicate that the influence of mass on TWLR differs significantly between acclimation temperatures. Total water loss rate was also positively affected by test

temperature, and this was revealed by a significant linear effect in the orthogonal polynomial contrast analysis (Table 5). In addition, the intercept method used in an attempt to extract respiratory water loss rate from total water loss rate proved ineffective as more than half the percentage of individuals in this study (i.e. 55%) displayed a negative value for respiratory water loss for one or more test temperatures (Table S2).

Table 4. Results of an orthogonal polynomial contrast analyses comparing the metabolic rate (log_{10} μW) of *Pringleophaga marioni* caterpillars at constant acclimation temperatures of 5°C, 10°C and 15°C.

	d.f.	SS	MS	*F*-value	*P*
acc	**2**	**0.269**	**0.135**	**3.099**	**0.0472**
tt	**3**	**17.182**	**5.727**	**131.866**	**< 0.0001**
m	**1**	**2.522**	**2.522**	**58.073**	**< 0.0001**
Contrast		Estimate	s.e.	*t*	*P*
acc linear		**-0.057**	**0.025**	**-2.314**	**0.0217**
acc quadratic		**-0.062**	**0.025**	**-2.472**	**0.0142**
tt linear		**0.573**	**0.029**	**19.880**	**< 0.0001**
tt quadratic		**-0.153**	**0.029**	**-5.354**	**< 0.0001**
m		**0.798**	**0.105**	**7.621**	**< 0.0001**

acc = acclimation; tt = test temperature; m = mass

Bold indicates significant effects

Table 5. Results of an orthogonal polynomial contrast analyses comparing the total water loss rate (log_{10} mg h^{-1}) of *Pringleophaga marioni* caterpillars at constant acclimation temperatures of 5°C, 10°C and 15°C.

	d.f.	SS	MS	*F*-value	*P*
acc	**2**	**0.658**	**0.329**	**3.798**	**0.024**
tt	**3**	**5.952**	**1.9841**	**22.901**	**< 0.0001**
m	**1**	**1.879**	**1.8793**	**21.692**	**< 0.0001**
acc*m	**2**	**0.698**	**0.3492**	**4.031**	**0.0192**
Contrast		Estimate	s.e.	*t*	*P*
acc linear		0.257	0.271	0.950	0.3434
acc quadratic		**0.600**	**0.233**	**2.577**	**0.0106**
tt linear		**0.357**	**0.041**	**8.772**	**<0.0001**
tt quadratic		-0.058	0.040	-1.448	0.1491
m		**0.767**	**0.157**	**4.899**	**<0.0001**
acc linear:m		0.327	0.288	1.133	0.2587
acc quadratic:m		**0.573**	**0.238**	**2.406**	**0.017**

acc = acclimation; tt = test temperature; m = mass

Bold indicates significant effects

When comparing the effects of acclimation on MR at each fluctuating acclimation temperature and its constant mean temperature (i.e. 5°C and 0-10°C, 10°C and 5-15°C, 15°C and 10-20°C), no significant interaction between acclimation and test temperature was found (i.e. result of maximal ANCOVA model with interaction term), indicating no variation in the slope. The only significant difference was in the intercepts between 10°C and 5-15°C (Table 6), where acclimation to the fluctuating temperature resulted in a lower MR at all test temperatures (Table 1, Fig. 2).

Table 6. Estimate outputs of analyses of covariance comparing the metabolic rate ($\log_{10}$ µW) of *Pringleophaga marioni* caterpillars at fluctuating and constant mean acclimation temperatures (i.e. 5°C and 0-10°C; 10°C and 5-15°C; and 15°C and 10-20°C).

Group		Estimate	s.e.	*t*	*P*
5°C and 0-10°C	intercept	1.749	0.146	11.941	<0.0001
	acc	0.001	0.041	0.031	0.9750
	tt	**0.063**	**0.005**	**12.484**	**<0.0001**
	m	**0.679**	**0.152**	**4.462**	**<0.0001**
10°C and 5-15°C	intercept	1.950	0.137	14.213	<0.0001
	acc	**-0.204**	**0.043**	**-4.775**	**<0.0001**
	tt	**0.069**	**0.005**	**13.306**	**<0.0001**
	m	**0.875**	**0.134**	**6.515**	**<0.0001**
15°C and 10-20°C	intercept	2.035	0.118	17.190	<0.0001
	acc	0.006	0.037	0.170	0.8650
	tt	**0.070**	**0.004**	**15.789**	**<0.0001**
	m	**1.120**	**0.131**	**8.555**	**<0.0001**

acc = acclimation; tt = test temperature; m = mass

Bold indicates significant effects

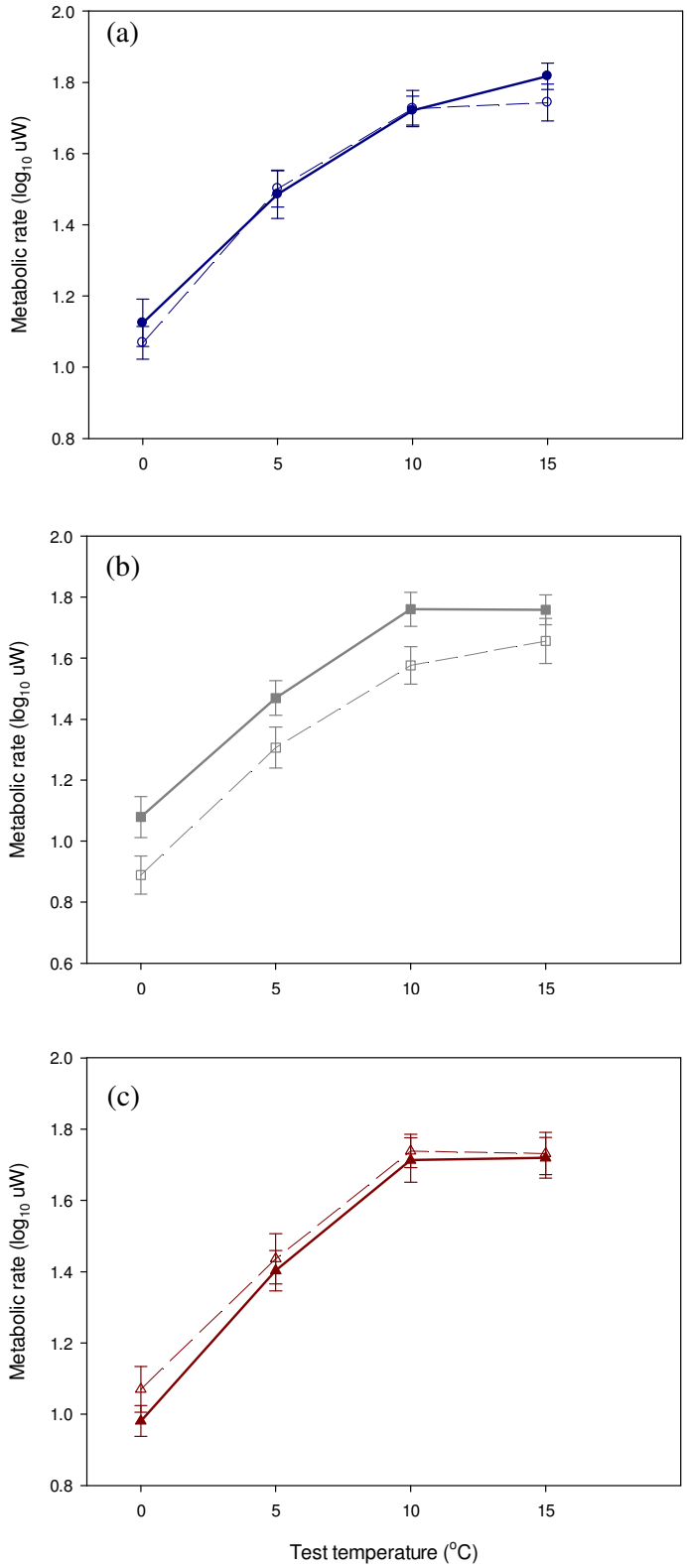

Fig. 2. The metabolic rate (log_{10} uW) of *Pringleophaga marioni* caterpillars at test temperatures of 0°C to 15°C at both fluctuating (indicated by the dotted line) and constant (indicated by the solid line) mean acclimation temperatures: 5°C and 0-10°C (**a**) are shown in blue, 10°C and 5-15°C (**b**) in grey, and 15°C and 10-20°C (**c**) in red. Means ± SE are shown.

Discussion

The primary goal of this study was to examine how temperature affects the metabolic rate of *P. marioni* caterpillars. I predicted that if metabolic rate is elevated at low temperatures, then support for the metabolic cold adaptation hypothesis may be provided. However, if water loss rate is significantly higher at low temperatures, then this may be evidence that caterpillars may depress their metabolic rates at high temperatures so as to conserve water. In addition, if fluctuating temperatures result in additional metabolic costs, I predicted that metabolic rate would be downregulated under fluctuating temperatures compared to constant mean temperatures.

Metabolic cold adaptation

Evidence for metabolic cold adaptation has been shown by either, a higher metabolic rate in cold temperature conditions compared to warmer conditions, or, reduced thermal sensitivity (i.e. lower slope) of the MR-T curve (Chown & Gaston 1999; Addo-Bediako *et al.* 2002; Irlich *et al.* 2009). No significant interaction was found between acclimation and test temperature, demonstrating no significant variation in slopes. However, a significant negative linear effect of acclimation on metabolic rate was found (Table 4), providing support for metabolic cold adaptation of a form that most resembles what has been termed the 'colder is better' hypothesis (Huey *et al.* 1999; e.g.'s Deere & Chown 2006; Terblanche *et al.* 2010). Caterpillars acclimated at the coldest constant temperature of 5°C had significantly higher metabolic rates compared to the warmer acclimation temperature of 15°C (Table 1; Fig. 1a). On Marion Island and other sub-Antarctic islands, some support for low temperature compensation has already been found for *P. marioni* caterpillars and other invertebrates (Sømme *et al.* 1989; Crafford & Chown 1993; Chown *et al.* 1997).

Water conservation

Partial metabolic cold adaptation suggests that selection may be acting to elevate metabolic rate in response to low temperatures (Clarke 2003). However, caterpillars may have also depressed their metabolic rates at the high acclimation treatment to conserve water (see Chown 2002; Davis *et al.* 2000). If so, then it was predicted that respiratory water loss rate would be lowest under high temperature conditions (Keister & Buck 1964). In insects, total water loss is dependent on respiration (i.e. *via* the spiracles), excretion, and transpiration through the cuticle (Gibbs 1998). *Pringleophaga marioni* caterpillars display continuous gas exchange cycles (Gibbs & Johnson 2004), as opposed to discontinuous gas exchange cycles

whereby respiratory water loss may be separated from cuticular water loss (Lighton 1994; 1996; Chown *et al.* 2006). Methods to extract respiratory water loss from total water loss have been developed for insects showing continuous gas exchange (e.g. Gibbs & Johnson 2004), but are still fairly controversial (see Gray & Chown 2008). In this study, the intercept method (Gibbs & Johnson 2004) was used to extract respiratory water loss rate from total water loss rate, but more than half the percentage of individuals showed negative values for respiratory water loss and the results are therefore of total water loss rates (Table S2). Acclimation had either no effect on total water loss rates (e.g. Gibbs *et al.* 1998; Terblanche *et al.* 2010) (Table 3), or a quadratic effect (Table 5; Fig. 1b), which indicates some variation in total water loss rate between the acclimation temperatures. Total water loss rate at the high temperature acclimation of 15°C was however not significantly lower than at 5°C. Thus, if respiratory water loss contributes significantly to total water loss in *P. marioni* caterpillars, then this may suggest that caterpillars do not depress their metabolic rates at high temperatures to conserve water.

Insects in mesic conditions are thought to display a weak selection for physiological mechanisms to limit water loss compared to those in more dry environments (see discussion in Zachariessen 1996; e.g. Terblanche *et al.* 2010). Nonetheless, both plants (*Arctotheca populifolia*) (Ripley *et al.* 1999) and invertebrates on Marion Island (weevil larvae, *Bothrometopus randi* (Chown & Van Drimmelen 1992) and *Embryonopsis halticella* caterpillars (Klok & Chown 1998)), have shown a considerable resistance to desiccation mainly as a result of very high wind speeds and limited boundary layers (Klok & Chown 1998). The lowland habitats of *P. marioni* are constantly wet (Klok & Chown 1997), and water loss is much lower in the soil, which may reduce the selection pressure to limit water loss (Convey 1996). However, apart from moist lowland habitats, populations of this species also occur in drier habitats, such as the fellfield, which are subjected to more extreme maximum temperatures than lowland ones (Klok & Chown 1997). In the latter habitats, *P. marioni* may very well face dessication stress, thus highlighting the importance of examining how this species may respond in dry conditions.

Jensen's inequality

Increases in thermal variability elevate metabolic rate due to Jensen's inequality. However, a high metabolic rate may prove costly, and ectotherms may respond to the effects of Jensen's inequality by a downregulating shift in the MR-T relationship (Ruel & Ayres 1999). A downregulation in metabolic rate in fluctuating conditions may be shown by either, a

decrease in the intercept, or, by decreasing thermal sensitivity. Acclimation to constant versus fluctuating temperatures either had no significant effect on metabolic rate, or metabolic rate was significantly lower in the fluctuating environment (indicated by a lower intercept at 5-15°C compared to 10°C) (Table 6; Fig. 2). Thus, caterpillars did not always consistently downregulate metabolic rate or the slope of the rate-temperature curve to avoid the consequences of Jensen's inequality. However, the significantly lower mean metabolic rate at 5-15°C compared to 10°C (Table 6; Fig. 2), could reflect a response to Jensen's inequality as a result of metabolic costs at the high temperature extremes. High temperature extremes may be stressful for organisms and a natural response would be a lowered metabolic rate (Lighton & Bartholomew 1988; Djawdan *et al.* 1997). Higher temperatures have been shown to have detrimental effects on caterpillar survival (Chapter 2), and this may be a consequence of the interaction between resource use (i.e. metabolic rate) and resource acquisition.

Conclusion

Overall, Marion Island is persistently cold with all year round precipitation (Le Roux & McGeoch 2008), thus selection towards adaptive strategies which compensate for low temperature conditions, as opposed to saving water, seems more likely to be operational. An elevated metabolic rate in cold conditions would allow for continuing growth (Sømme & Block 1991) and a year-long life cycle duration for *P. marioni* (Haupt *et al.* 2013) suggests that caterpillars may benefit by maintaining high metabolic rates which may allow feeding and thus development to be completed within this time.

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Supplementary material

Table S1.1. Results from a Shapiro-Wilk's test for normality and Levene's test for homogeneity of variances for metabolic rate (log_{10} µW) of *Pringleophaga marioni* at constant (const.) acclimation temperatures of 5°C, 10°C and 15°C, and fluctuating (fluc.) acclimation temperatures of 0-10°C, 5-15°C and 10-20°C.

acclimation temperature	Shapiro-Wilk's	Levene's
5°C	W = 0.9543, p = 0.0107	
10°C	W = 0.9738, p = 0.1371	
15°C	W = 0.9794, p = 0.2847	
const. (5, 10 & 15°C)	W = 0.9801, p = 0.1045	F = 0.001, d.f. = 1,106, p = 0.9818
0-10°C	W = 0.9636, p = 0.0353	
5-15°C	W = 0.9821, p = 0.3986	
10-20°C	W = 0.9667, p = 0.0537	
const. vs. fluc. (5 & 0-10°C)	W = 0.9801, p = 0.1045	F = 0.001, d.f. = 1,142, p = 0.9767
const. vs. fluc. (10 & 5-15°C)	W = 0.9870, p = 0.3816	F = 0.08, d.f. = 1,106, p = 0.7843
const. vs. fluc. (15 & 10-20°C)	W = 0.9836, p = 0.2054	F = 0.03, d.f. = 1,106, p = 0.8532

Table S1.2. Results from a Shapiro-Wilk's test for normality and Levene's test for homogeneity of variances for total water loss rate (mg h^{-1}) of *Pringleophaga marioni* at constant acclimation temperatures of 5°C, 10°C and 15°C.

acclimation temperature	Shapiro-Wilk's	Levene's
5°C	W = 0.92796, p = 0.0005	
10°C	W = 0.9234, p = 0.0003	
15°C	W = 0.9656, p = 0.0458	
constant temperatures (5°C, 10°C and 15°C)		F = 0.011, d.f. = 2, 213, p = 0.9895

Table S2. Calculations of respiratory water loss rate (RWLR) and cuticular water loss rate (CWLR) from total water loss rate (TWLR) using the VH_2O - VCO_2 y intercept method by Gibbs & Johnson (2004), whereby VH_2O is regressed over VCO_2. The example given below is of 12 out of the 19 individuals at the 5°C acclimation temperature for which negative values were obtained for respiratory water loss at one or more test temperatures (TT) using the above method. These numbers were 15 and 10 individuals for 10°C and 15°C respectively, proving this method ineffective for calculating RWLR.

Individual	TT (°C)	TWLR (mg h^{-1})	slope	intercept/CWLR (mg h^{-1})	r^2	*F*	*P*	RWLR (TWLR - CWLR) (mg h^{-1})
5.19	0	1.123	-15.655	1.232	0.026	7.050	0.008	**-0.109**
5.19	5	2.422	10.453	2.313	0.051	15.925	< 0.0001	0.110
5.19	10	3.496	5.932	3.430	0.097	27.145	< 0.0001	0.066
5.19	15	5.147	8.045	4.998	0.059	30.957	< 0.0001	0.149
5.20.	0	0.569	-0.184	0.569	0.0003	0.109	0.4	**-0.0002**
5.20.	5	1.631	13.139	1.560	0.017	7.093	0.008	0.071
5.20.	10	2.393	0.817	2.386	0.007	4.228	0.040	0.007
5.20.	15	3.465	-1.202	3.480	0.013	6.529	0.011	**-0.015**
5.21	0	1.728	-3.091	1.740	0.001	0.359	0.450	**-0.012**
5.21	5	1.824	169.803	0.373	0.820	1021.258	< 0.0001	1.451
5.21	10	4.654	23.852	3.941	0.106	35.511	< 0.0001	0.713
5.21	15	4.848	21.487	4.567	0.414	242.163	< 0.0001	0.281
5.23	0	1.221	-1.790	1.275	0.001	0.540	0.463	**-0.054**
5.23	5	3.693	29951.900	1.660	0.690	685.634	< 0.0001	2.033
5.23	10	6.126	-232.418	8.407	0.239	151.160	< 0.0001	**-2.281**
5.23	15	7.435	46.831	7.013	0.273	225.320	< 0.0001	0.422

table S2 cont.

5.24	0	1.956	-7.900	1.974	0.012	4.171	0.004	**-0.018**
5.24	5	2.723	8.755	2.686	0.193	61.558	< 0.0001	0.037
5.24	10	3.666	6.246	3.456	0.142	50.208	< 0.0001	0.210
5.24	15	5.711	71.478	4.867	0.109	73.411	< 0.0001	0.844
5.25	0	0.098	-34.691	0.133	0.036	10.795	0.001	**-0.035**
5.25	5	2.713	44.437	2.487	0.039	17.217	< 0.0001	0.226
5.25	10	4.134	534.378	0.824	0.526	351.819	< 0.0001	3.310
5.25	15	5.717	23.897	5.449	0.049	21.001	< 0.0001	0.268
5.29	0	2.308	11.324	2.245	0.005	1.749	0.187	0.063
5.29	5	1.928	8.540	1.844	0.109	63.429	< 0.0001	0.084
5.29	10	2.772	18.394	2.159	0.579	422.291	< 0.0001	0.613
5.29	15	4.271	-7.154	4.505	0.049	16.559	< 0.0001	**-0.235**
5.31	0	1.382	-23.669	1.425	0.059	26.730	< 0.0001	**-0.044**
5.31	5	2.490	-5.233	2.504	0.004	1.774	0.184	**-0.013**
5.31	10	2.859	-17.760	2.940	0.051	16.475	< 0.0001	**-0.081**
5.31	15	4.016	6.069	3.961	0.193	88.973	< 0.0001	0.055
5.34	0	4.825	-2.152	5.871	0.026	8.127	0.0047	**-1.046**
5.34	5	3.673	24.970	3.615	0.022	13.164	0.0003	0.058
5.34	10	10.243	25.898	9.254	0.008	3.001	0.0840	0.989
5.34	15	15.447	789.688	5.264	0.560	563.220	< 0.0001	10.183
5.37	0	3.213	8.307	3.204	0.052	18.703	< 0.0001	0.009
5.37	5	4.452	27.655	4.355	0.014	4.197	0.041	0.097
5.37	10	10.687	-512.444	15.390	0.359	137.356	< 0.0001	**-4.703**

table S2 cont.

5.37	15	7.843	444.763	5.111	0.433	270.062	< 0.0001	2.732
5.38	0	3.901	55.476	3.696	0.025	8.267	< 0.0001	0.206
5.38	5	4.929	173.857	3.312	0.194	60.067	< 0.0001	1.617
5.38	10	7.959	153.257	5.858	0.255	205.204	< 0.0001	2.100
5.38	15	10.835	-35.379	11.401	0.068	33.769	< 0.0001	**-0.566**
5.40.	0	6.462	73.567	6.367	0.093	31.013	< 0.0001	0.095
5.40.	5	6.241	30.626	6.131	0.005	2.363	0.125	0.110
5.40.	10	5.795	8.629	5.729	0.022	12.550	0.000	0.067
5.40.	15	7.319	-8.480	7.382	0.003	1.417	0.234	**-0.063**

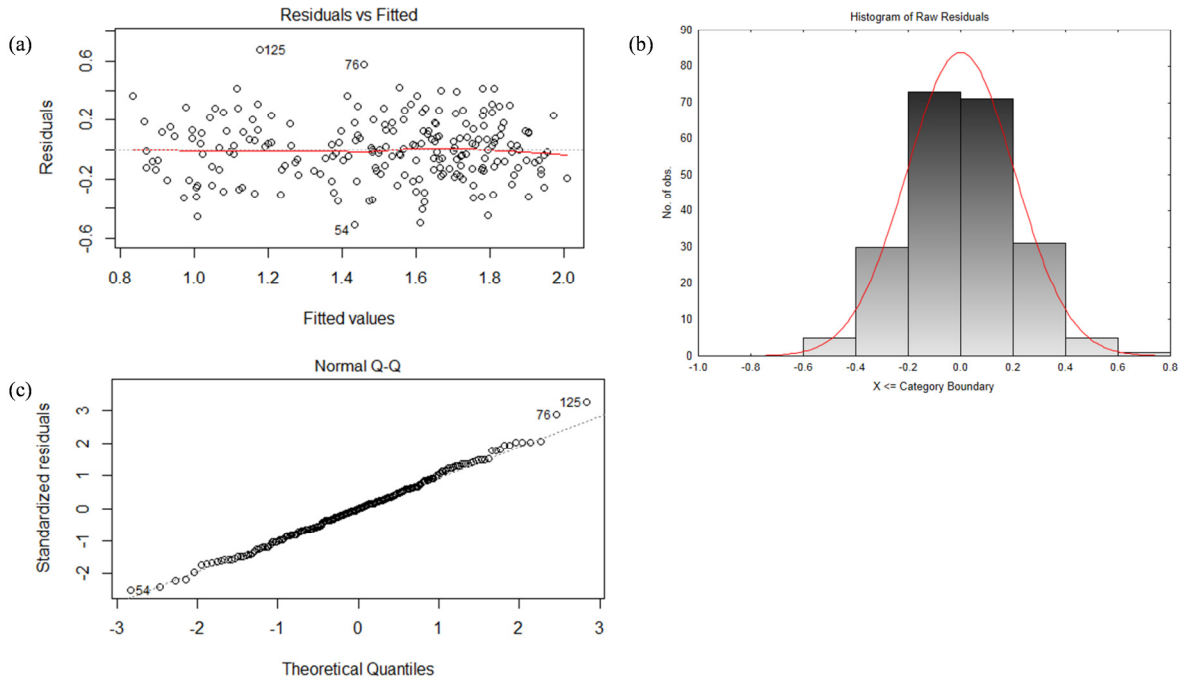


Fig. S1. Metabolic rate ($\log_{10}$ µW) at constant acclimation temperatures (5°C, 10°C and 15°C), a plot of (a) residuals versus fitted values, (b) a histogram of residuals, and (c) a normal probability plot of residuals.

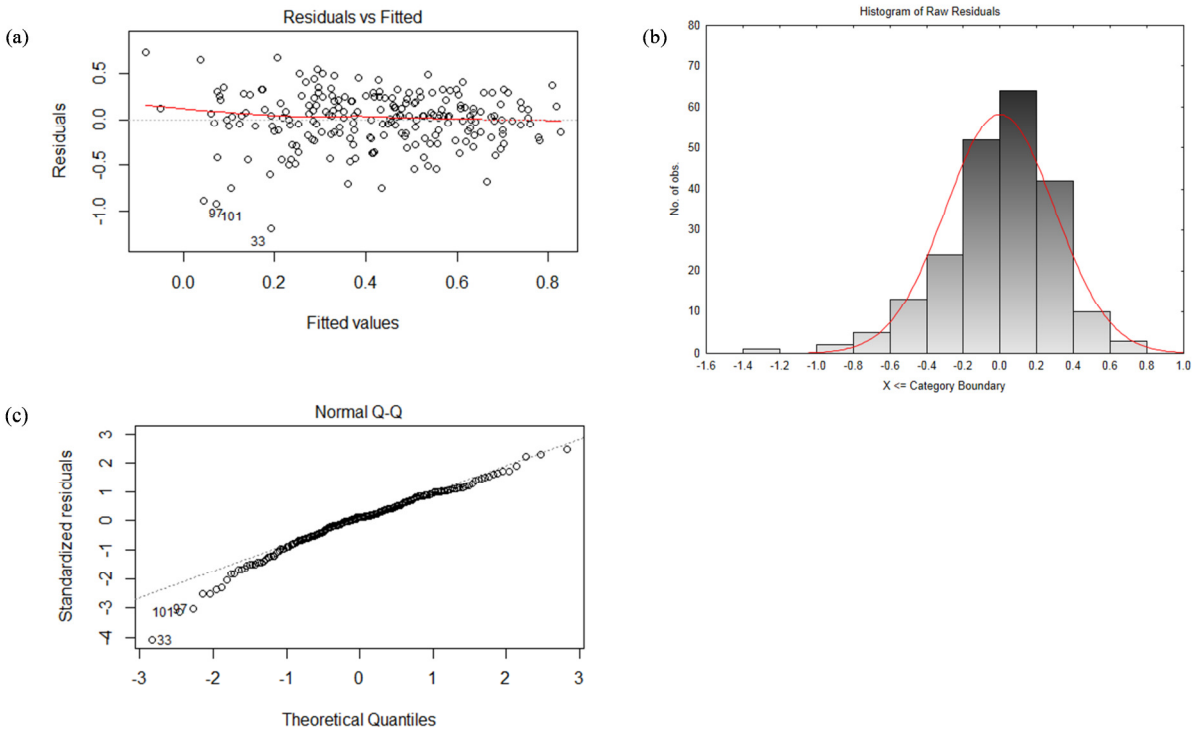


Fig. S2. Total water loss rate ($\log_{10}$ mg h^{-1}) at constant acclimation temperatures (5°C, 10°C and 15°C), a plot of (a) residuals versus fitted values, (b) a histogram of residuals, and (c) a normal probability plot of residuals.

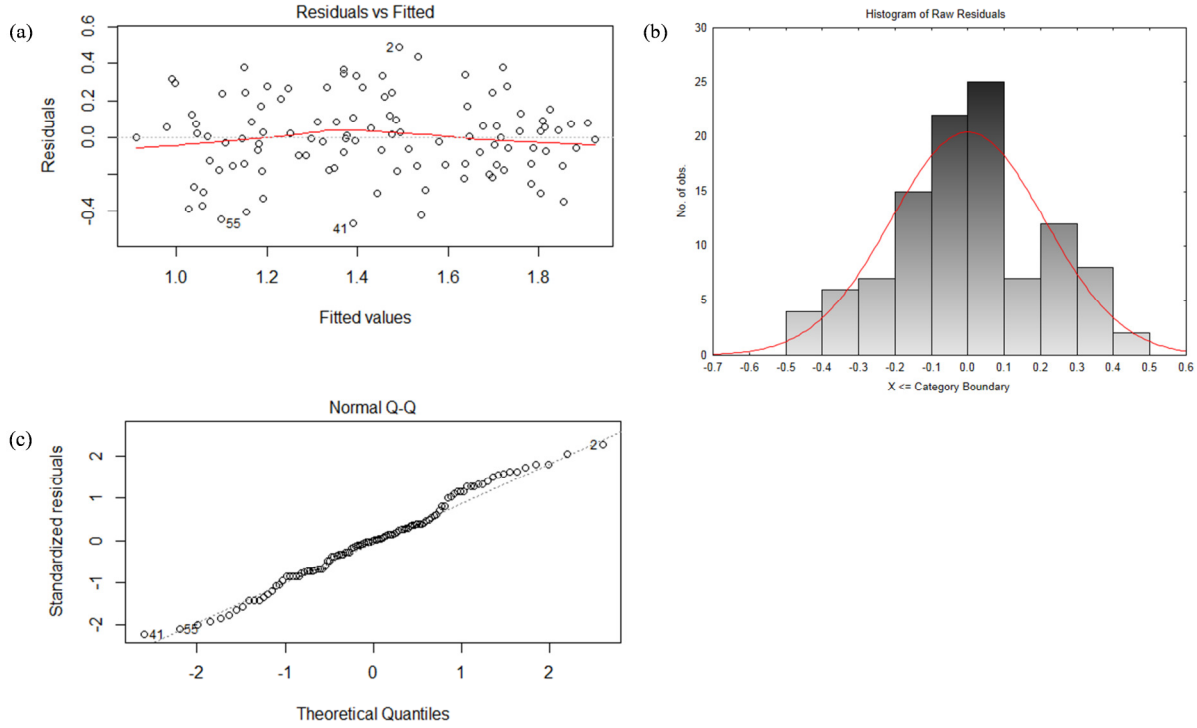


Fig. S3a. Metabolic rate ($\log_{10}$ µW) at 5°C and 0-10°C, a plot of (a) residuals versus fitted values, (b) a histogram of residuals, and (c) a normal probability plot of residuals.

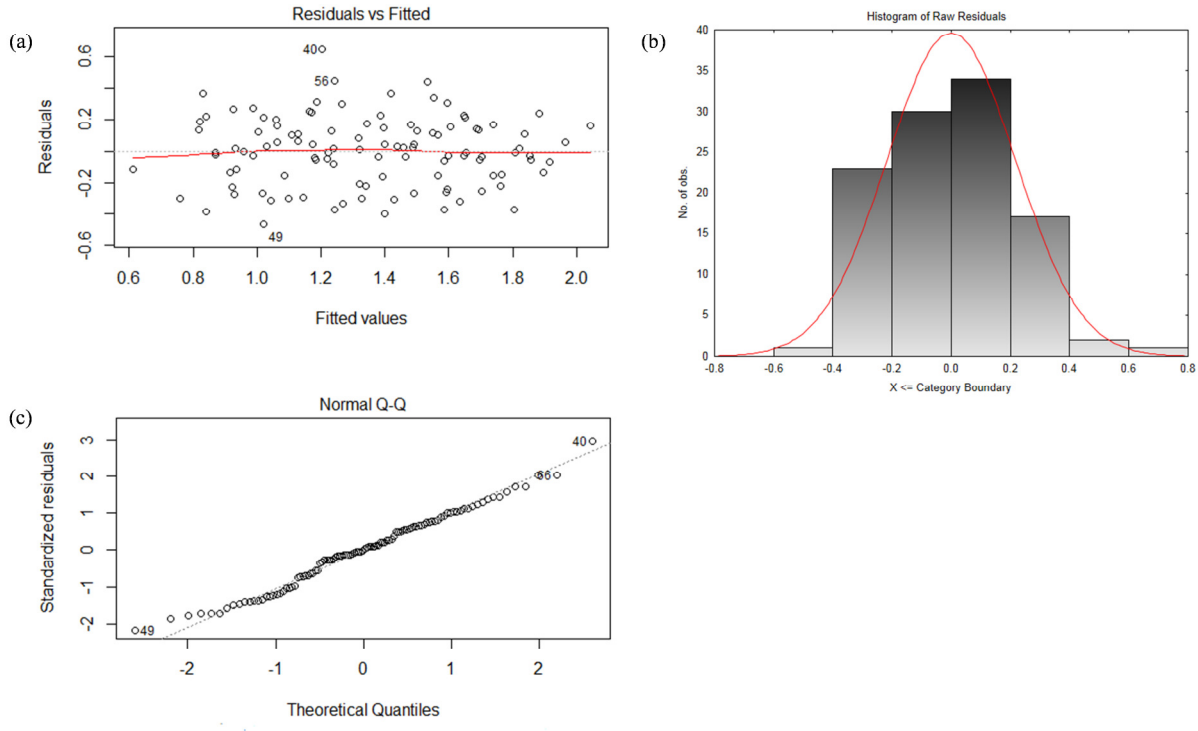


Fig. S3b. Metabolic rate ($\log_{10}$ µW) at 10°C and 5-15°C, a plot of (a) residuals versus fitted values, (b) a histogram of residuals, and (c) a normal probability plot of residuals.

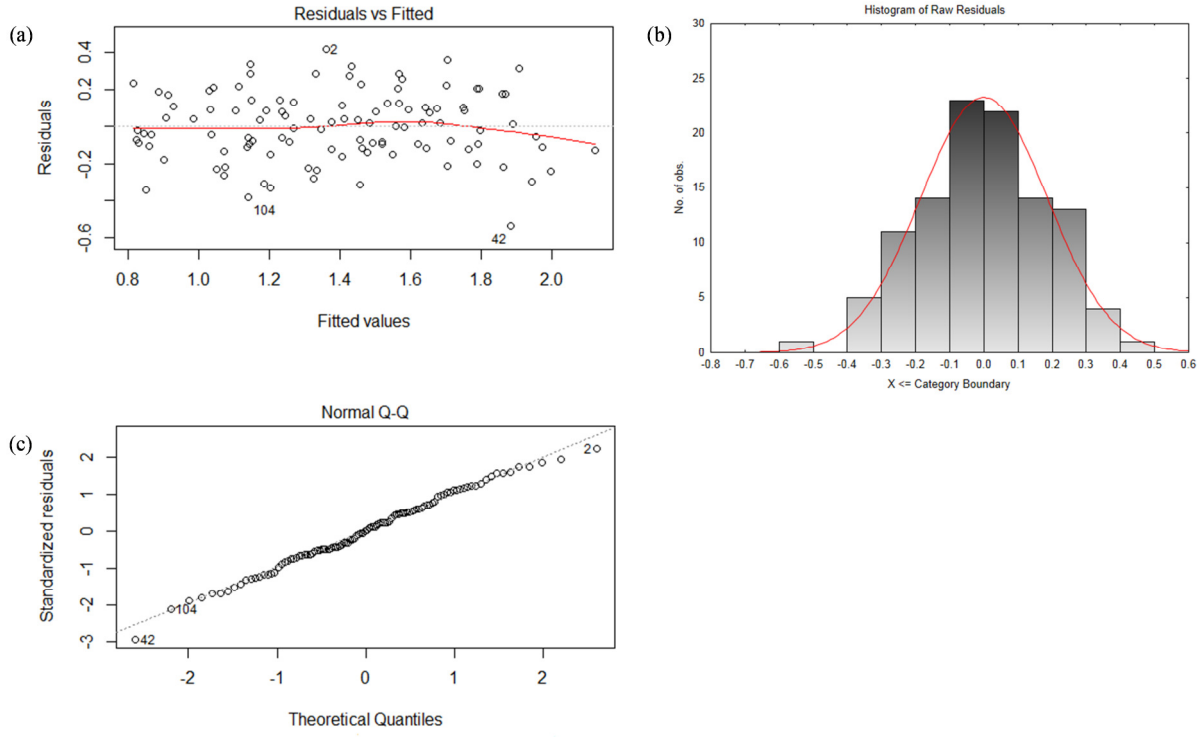
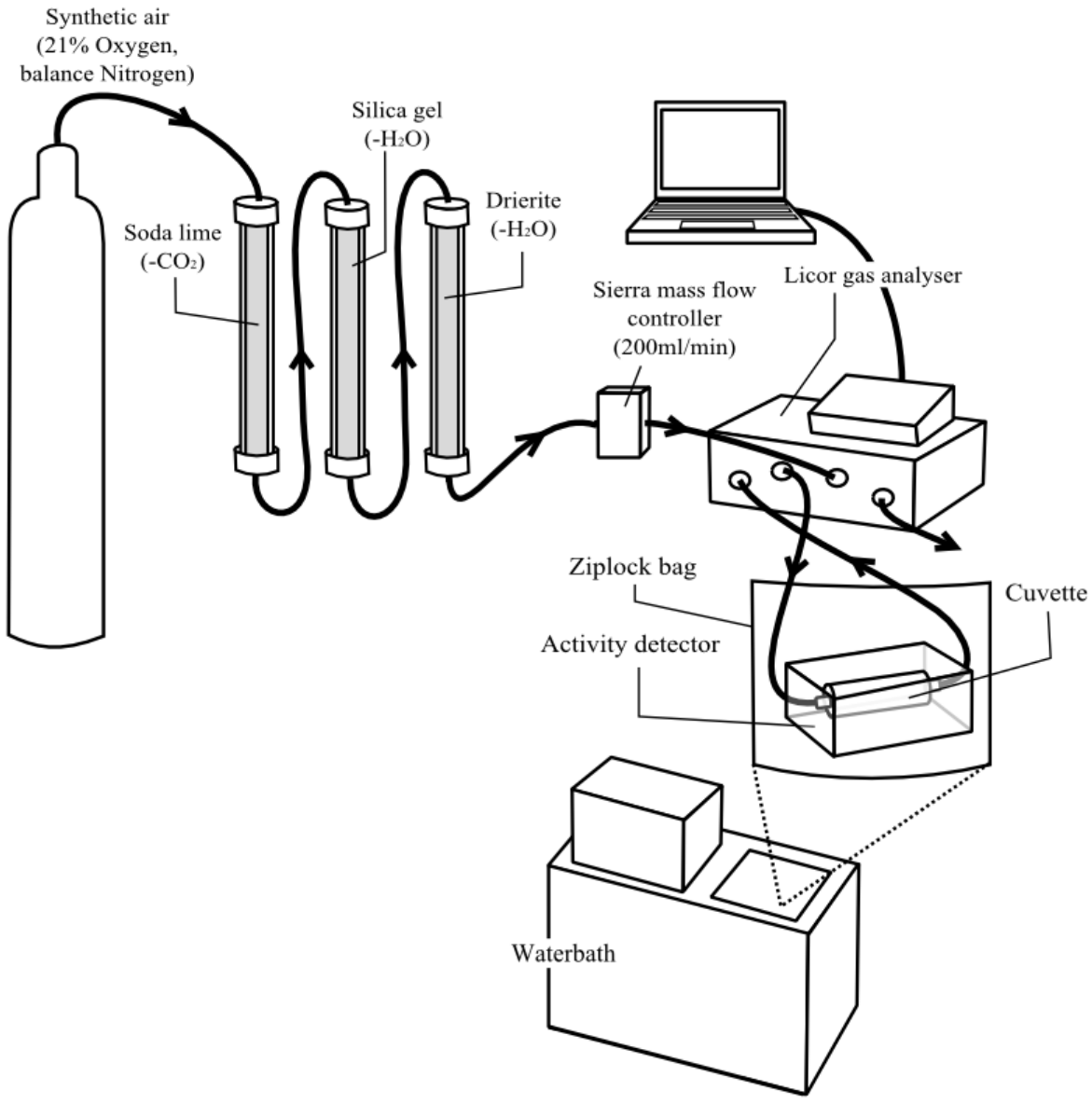


Fig. S3c. (a) Metabolic rate ($\log_{10}$ µW) at 15°C and 10-20°C, a plot of (a) residuals versus fitted values, (b) a histogram of residuals, and (c) a normal probability plot of residuals.

Appendices

Appendix A

Schematic diagram showing the flow of air through the respirometry set-up. From the gas cylinder, air flowed via plastic tubing to cylinders of soda lime, silica gel and drierite (to remove CO_2 and H_2O), through the Sierra mass flow controller (set at 200 ml min^{-1}) into the LiCor (gas analyser) where initial CO_2 and H_2O readings were taken. Air flowed from the Licor into the cuvette in which the caterpillar was held on an activity detector in a ziplock bag placed inside a water bath (set at the desired test temperature). Final readings of CO_2 and H_2O were measured when air flowed back into the gas analyser.



Chapter 5 – Thermal preference and performance in a sub-Antarctic caterpillar: a test of multiple hypotheses [1]

Flightless moth caterpillar, *Pringleophaga marioni*.

Photo credit: C. Janion-Scheepers

[1] To be submitted as: Haupt, T.M., Sinclair, B.J. & Chown, S.L. Thermal preference and performance in a sub-Antarctic caterpillar: a test of multiple hypotheses. *The Journal of Experimental Biology.*

Abstract

Physiological ecologists have long assumed that thermoregulatory behaviour and thermal physiology will coincide over time. The reason behind this concordance is thought to be that if organisms choose temperatures that maximise performance, then greater fitness benefits will be achieved. Termed coadaptation, this hypothesis predicts that an animal's preferred body temperature will correspond to its optimum temperature of performance. Consequently, many studies document thermal preferences that match or are close to temperatures that maximise performance. Nonetheless, mismatches between thermal preference and thermal optima do occur, and several hypotheses have been proposed to explain this departure from coadaptation. In this study, I used a strong inference approach to examine the relationship between thermal preference and locomotor performance in sub-Antarctic *Pringleophaga marioni* caterpillars. Specifically, if these traits are closely matched (i.e. coadaption), and if not, hypotheses that may explain this departure. In brief, these are, (i) 'suboptimal is optimal', performance decreases rapidly once optimum temperatures are reached (i.e. highly asymmetric performance curves), and preference should be for lower temperatures; (ii) 'thermodynamic effects hypothesis', in the presence of a thermodynamic effect, natural selection should favour a thermal optimum that exceeds mean body temperature; (iii) 'trait variation', if optimum temperatures vary among physiological processes, then no single thermal preference will optimize all systems; and lastly (iv) the 'immobility hypothesis', preference will be low if organisms are trapped at the lower end of laboratory gradients. Compared to the optimal movement temperature (*c.* 23°C), thermal preference was far lower at *c.* 8°C. After accounting for the possibility that caterpillars may have been trapped at the cool end of the thermal gradient, the low degree of asymmetry observed in the performance curves, and the finding that acclimation to high temperatures did not result in maximal performance, all, but one of the above hypotheses (i.e. 'trait variation') was rejected. The thermal preference of *P. marioni* caterpillars more closely resembled temperatures at which survival is high (5-10°C), or where feeding is optimal (10°C), suggesting that thermal preference may differ depending on the physiological trait examined.

Keywords: coadaptation – suboptimal – locomotion - performance curves – thermal preference

Introduction

Because temperature is by far the most important environmental factor affecting most biological processes in organisms, a common assumption is that preference will be for body temperatures at which performance is optimal (Angilletta *et al.* 2006). Such coadaptation implies that thermoregulatory behaviour and thermal physiology should converge over time (reviewed in Angilletta *et al.* 2002a; Angilletta *et al.* 2006; Angilletta 2009). If fitness is positively related to performance, then organisms that prefer optimum temperatures should have an advantage over those that do not (Huey & Bennet 1987; Angilletta *et al.* 2002a; Huey *et al.* 2003). In consequence, natural selection should result in similarity between the two traits because this should maximise Darwinian fitness (Martin & Huey 2008; Anderson *et al.* 2011).

By measuring the thermal sensitivity of sprint speed in *Anolis* lizards, Van Berkum (1986) showed that the optimum temperature for sprint speed was positively correlated with field body temperatures. Similarly, Huey & Bennet (1987) measured the thermal sensitivity of sprint speed as well as thermal preference of 11 species of Australian skinks, and showed partial coadaptation between these traits, thus providing one of the first quantitative tests of coadaptation. Since then, evidence for coadaptation has come from investigations of reptiles (e.g. Garland *et al.* 1991; Huey & Kingsolver 1993; Bauwens *et al.* 1995; Angilletta *et al.* 2002b; Kubisch *et al.* 2011), insects (Sanford & Tschinkel 1993; Forsman 1999; Calabria *et al.* 2012), and other ectotherm taxa (Anderson *et al.* 2011; Khan & Herbert 2012). Indeed, such coadaptation of preferred and optimal temperatures has been and remains a foundational concept in thermal physiology (Huey & Stevenson 1979; Huey & Bennet 1987; Huey & Kingsolver 1993; Angilletta *et al*. 2006; Angilletta 2009; Clusella-Trullas *et al*. 2011).

Although there is significant evidence for coadaptation, there are documented examples of mismatches between thermoregulatory behaviour and thermal physiology. For example, several studies examining the locomotor performance of lizards have found that optimal performance is achieved at a higher temperature than preferred body temperatures (e.g. Bennet 1980; Huey & Bennet 1987; Huey *et al*. 1989; Van Damme *et al*. 1989; Du *et al.* 2000; Lailvaux *et al.* 2003; Zang & Ji 2004; review in Martin & Huey 2008; Fernandez *et al.* 2011). Similar findings for population growth have been made for insects (e.g. Smith 1965; Langer & Young 1976; Allsopp *et al.* 1980; Allsopp 1981; White 1987; Jian *et al.* 2002) and other ectotherms (Åkesson 1976; Zhang & Lefcort 1991; Prevedelli & Simonini 2001; Jia *et al.* 2002; Tepler *et al.* 2011).

Several hypotheses have been proposed to explain this departure from coadaptation, and specifically the cases where optimum temperatures that are higher than preferred temperatures. First, Martin & Huey (2008) suggested that preferred temperatures should be lower than optimum temperatures (i.e. 'suboptimal is optimal') because asymmetric performance curves mean that performance decreases rapidly once the optimum temperature is reached (Huey & Stevenson 1979; Huey & Kingsolver 1989). Thus, preference should be towards lower temperatures such that the risk of reduced performance and presumably fitness is minimized, especially in organisms that are imperfect thermoregulators (Martin & Huey 2008).

Second, Asbury & Angilletta (2010) demonstrated that, in the presence of a thermodynamic effect (i.e. poorer performance at low temperatures because biochemical reactions proceed more slowly, (Frazier *et al*. 2006; Angilletta *et al*. 2010)), natural selection should favour a thermal optimum that exceeds mean body temperature. Moreover, the optimal mismatch between the mean temperature and the thermal optimum should increase with increasing variation in body temperature. Asbury & Angilletta (2010) made clear that selection driven by a thermodynamic effect could explain differences between thermoregulatory behaviour and thermal physiology. This is particularly true for the large differences (*c*. 8°C) found in some studies between preferred temperature and the thermal optimum. This might be termed the 'thermodynamic effects hypothesis'.

A third hypothesis may be termed the 'trait variation' hypothesis. That is, if optimum temperatures vary among physiological processes, then no single thermal preference will optimize all systems (Huey & Stevenson 1979). In consequence, thermal preference may depend on where the major constraints for fitness lie under a given set of conditions. For example, when nutrients are plentiful, preference for high temperature in migratory locusts favours maximal growth rather than efficient utilization of nutrients, but when nutrients are limited, the preferred temperature declines to maximize efficiency (Miller *et al*. 2009; Coggan *et al*. 2011; Clissold *et al.* 2013). This hypothesis reflects the more general one that there may be differential effects of temperature on individual traits and on overall fitness, and that understanding the relationships between the adaptive value of particular trait values and overall fitness is important (Kingsolver & Woods 1997; Woods & Harrison 2002).

Finally, what may be termed the 'immobility hypothesis' explains the frequent occurrence of low preferred temperatures, compared with optimum temperatures, as an outcome of experimental design. Body temperatures and therefore speeds of movement of small ectotherms match temperatures at a given location on the gradient, thus making it

difficult to distinguish between thermal preference and thermal dependence of movement. In addition, studies examining thermal preferences on laboratory gradients may use temperatures that are too close to the critical thermal minimum temperature, and small ectotherms may become trapped at the lower end of the thermal gradient, thus biasing optimal temperature estimates downwards (Dillon *et al.* 2012).

Although empirical evidence has been provided in support of most of these hypotheses, they have rarely been the subject of simultaneous consideration in the form of the strong inference approach recommended for investigations of alternative explanations for variations in thermal performance under various circumstances (e.g. Platt 1964; Huey et al. 1999). Here, I do so using a species for which considerable knowledge about its thermal physiology, growth and environment are available – caterpillars of the flightless sub-Antarctic moth, *Pringleophaga marioni* Viette (Klok & Chown 1997; Sinclair & Chown 2003; Sinclair *et al*. 2004; Sinclair & Chown 2005; 2006). Specifically, I examined the relationship between thermal preference (T_{pref}) and the thermal optimum (T_{opt}) for locomotion. In effect I posed a series of questions that would enable me to distinguish these hypotheses. First, has the experimental design perhaps resulted in underestimation of the thermal preference? If this is the case, an adjustment in design or analysis should be made to account for this effect. Second, are T_{opt} and T_{pref} indistinguishable? If these traits are similar, then support for the coadaptation hypothesis may be provided. However, if they are different, is the magnitude of this difference relatively small, and is the performance curve asymmetric, thus providing support for the 'suboptimal is optimal" hypothesis? Alternatively, does variation in performance curves following exposure to different acclimation regimes accord with the expectations of a thermodynamic effect (i.e. the 'thermodynamic effects hypothesis)'? Lastly, if none of the previous hypotheses are supported, does thermal preference align with some other performance measure that may be more significant for a detritivorous caterpillar? If so, support for the 'trait variation' hypothesis may be provided.

Materials and methods

Study site and species

Pringleophaga marioni (Lepidoptera: Tineidae) occurs in virtually all habitats on the sub-Antarctic Marion and Prince Edward islands (46.9°S, 36.7°E) (Crafford *et al*. 1986; Haupt *et al*. 2013). Caterpillars have a critical thermal minimum (CTmin) temperature range of -1.6°C to 0.1°C and a critical thermal maximum temperature (CTmax) range of 37.7°C to 38.7°C (Klok & Chown 1997). Marion Island has a low mean annual air temperature of 6.5°C with

relatively stable ambient air temperatures ranging from 2°C in summer to 7°C in winter and a total annual precipitation of 1900 mm (Le Roux & McGeoch 2008). At low altitudes (4-6 m a.s.l), soil microhabitat temperatures are 6.1 ± 2.7 (°C) (mean ± s.d.), with minimum and maximum temperatures at -1.1°C and 22.5°C respectively. At high altitudes (750 m a.s.l) where *P. marioni* are also found (Crafford *et al.* 1986), mean soil temperatures are 2.1 ± 3.8 (°C) (mean ± s.d.) with minimum and maximum temperatures at -11.2°C and 24.5°C respectively (S.L. Chown, unpublished data).

Collection and acclimation

This study was undertaken in the laboratory on Marion Island during relief voyages between 2010 and 2012. Caterpillars were collected from abandoned wandering albatross nests (*Diomedea exulans*), where they occur in high numbers (Sinclair & Chown 2006), and returned to the laboratory within six hours of collection. Individuals were placed in petri dishes filled with albatross nest material, which served as both refuge and food (Haupt *et al*. 2013). Keeping individuals separate was necessary to avoid cannibalism (French & Smith 1983). To measure if exposure to different acclimation regimes results in demonstration of a thermodynamic effect, caterpillars were held for seven days in incubators (MIR 154, Sanyo, Osaka, Japan, accurate to ± 0.5°C) set at 0°C, 5°C, 10°C and 15°C. The timing of acclimation period was based on previous trials showing acclimation responses within a week for this species (Sinclair & Chown 2003) and for insects generally (Weldon *et al*. 2011). The effects of variable temperature regimes were also examined by acclimating caterpillars to a fluctuating temperature of 5-15°C. Temperatures inside the chambers were measured with iButton Thermochron dataloggers (means: 0 = 0.6°C, 5 = 5.4°C, 10 = 10.0°C, 15 = 15.7°C, 5-15 = 4.1-14.7°C). Constant temperatures fall within the soil microhabitat temperature range for this species on Marion Island (Chown & Crafford 1992; S.L. Chown, unpublished data) and the fluctuating temperature simulates conditions within wandering albatross nests (Sinclair & Chown 2006). A group of caterpillars were also kept at 5°C for only three days, and these field-fresh individuals served as a control (Deere & Chown 2006). At the beginning of each experiment, individuals were weighed (± 0.5 mg) using a Mettler AE163 balance (Mettler-Toledo Ltd, EngNet, South Africa).

Thermal preference trials

Thermal preference was first determined at temperatures ranging from *c*. 0-15°C, i.e. microclimate temperatures on Marion Island (Chown & Crafford 1992). Because caterpillars

showed no defined preference on this gradient (Table S1), this experiment was then repeated on a gradient of *c*. 0-30°C using a different group of individuals. Experiments were conducted on a temperature gradient (Length 75 cm) with temperatures controlled at both ends using water baths (LTC 12, Grant Instruments Ltd., Cambridge, UK) (see Appendix A for experimental set-up). The temperatures of the baths were set to obtain the required temperature gradients. Temperature along the gradient was measured and recorded every 5 s using eight evenly-spaced, Type T 40 gauge thermocouples connected to an eight channel SQ800 Grant Squirrel data logger (Grant Instruments Ltd, Cambridge, U.K.). Black plastic containers serving as refuges were placed along the gradient at intervals corresponding with the thermocouple positions following Marais & Chown (2008). At the start of each trial, an individual was placed in the centre of the gradient. A black plastic bag was then placed over the apparatus to ensure that the gradient was darkened and caterpillars were not choosing temperatures that were biased by the influence of light. After one hour, the temperature at the position of where the individual was found along the gradient was recorded. In another experiment (using a different group of individuals), the temperature of the gradient was set to *c.* 10°C, i.e. no variance in temperature. This constant temperature gradient provides a null model against which to confirm that caterpillars show a thermal preference as opposed to favouring particular ends of the gradient (Anderson *et al.* 2007).

Locomotor performance trials

The locomotion speed of individual caterpillars (n = 28 per acclimation temperature) was measured using a temperature-controlled walking stage (see Appendix B). A water bath (Grant LTC 12, Instruments Ltd., Cambridge, UK) controlled the temperature of the stage which was measured by a digital thermometer (CHY 507, Firemate Co, Taiwan) through Type-K thermocouples. To avoid heat shock affecting performance at lower temperatures (Lachenicht *et al.* 2010), individuals were examined first at randomized test temperatures of 0°C, 5°C, 10°C, 15°C and 20°C, and then at high temperatures of 25°C, 30°C and 35°C as these temperatures approach the critical thermal maximum temperature of 38°C for *P. marioni*. At the start of each experiment, the caterpillar was placed in the centre of the walking stage and held under a plastic container for four minutes to equilibrate to the temperature being tested. After this time, it was released and when it moved without faltering, the distance that the head capsule moved over a 20 s period was recorded. The trial was repeated three times in succession. The longest distance recorded was used in the analyses, because lower values may reflect an individual's unwillingness to move rather than

its inability to move faster (Huey & Bennet 1987; Angilletta *et al.* 2002b). Between different temperature trials, individual caterpillars were returned to the petri dish they were taken from and held at their acclimation temperature for a minimum of one hour before the next temperature trial.

From these measurements, the key performance traits of optimum temperature (T_{opt}), maximum speed at the optimum temperature (U_{max}), and performance breadth (the index of the breadth of the curve, T_{br}) were obtained. The optimum temperature and maximum speed were chosen from the experimental data (i.e. the test temperature with the greatest speed) (Gilchrist 1996), and these values were used to calculate T_{br} for each individual using Gilchrist's (1996) formula:

$$T_{br} = \sqrt{\sum \left[\frac{u_i (T_i - T_{opt})}{u_{\max}} \right]^2}$$

where T_{opt} is the temperature at which an individual moved the fastest, U_{max} is the maximum speed at T_{opt}, and U_i is the speed at T_i, i.e. speed at a given test temperature.

Data analyses

Regression analyses did not show a relationship between body mass and each performance trait or thermal preference ($p > 0.05$ in all cases, results not shown), indicating that mass was not responsible for any variation observed and it was therefore not included as a covariate in any of the analyses. In consequence, analyses proceeded as follows. First, I tested whether T_{pref} was not biased by experimental design (i.e. 'immobility hypothesis'). The median thermal preference for each acclimation temperature on the *c*. 0-30°C gradient was calculated using all individuals. To be certain that individuals were not trapped at their CTmin temperatures, the median thermal preference was also calculated after excluding individuals that preferred temperatures below 0.2°C, since the CTmin range for *P. marioni* lies between -1.6°C and 0.1°C. For each treatment group (i.e. each acclimation temperature and field fresh individuals), a Wilcoxon rank-sum test (because of non-normal data), as implemented in R.3.0.0 was used to test whether there were any significant differences in thermal preference when individuals with preferences close to or within the range of CTmin values were excluded.

To determine if T_{opt} and T_{pref} are indistinguishable (i.e. coadapted), the medians of T_{opt} and T_{pref} for each treatment group were compared using Wilcoxon rank-sum tests (because of

non-normal data). In addition, to determine how asymmetric the performance curves were, the degree of asymmetry was calculated for each individual using the following equation as in Martin & Huey (2008):

$$\text{asymmetry} = \frac{2\text{Topt} - \text{Tmax} - \text{Tmin}}{\text{Tmax} - \text{Tmin}}$$

where Topt is the temperature at which an individual moved the fastest, and Tmax and Tmin are the upper and lower limiting temperatures for performance respectively (Martin & Huey 2008). The latter values were extracted from the experimental data by identifying the minimum and maximum temperatures at which locomotion was slowest (i.e. Tmin and Tmax respectively).

To test the 'thermodynamic effect hypothesis', ordered factorial ANOVAs (analysis of variance) with orthogonal polynomial contrasts as in Huey *et al.* (1999) were used to distinguish whether "warmer is better" from the alternative acclimation hypotheses (see Deere & Chown 2006). These analyses compared T_{opt}, U_{max} and T_{br} between the constant acclimation temperatures of 0°C, 5°C, 10°C, and 15°C, as these temperatures were ordered. Orthogonal polynomial contrast analyses require strict adherence to the assumptions of ANOVA, which are, normally distributed residuals, homogeneity of variance and a balanced design (Huey *et al.* 1999). Shapiro-Wilk's tests showed instances of non-normality, but Levene's tests and plots of the residuals indicated normality and homogeneity of variances (Table S2; Fig S2).

In addition, to examine the effects of the fluctuating acclimation temperature on performance, an ANOVA was used to compare performance traits between 5-15°C and 10°C (i.e. the closest comparable constant temperature). Similarly, field-fresh individuals were compared with those that were subjected to acclimation. The effect of acclimation on thermal preference was also examined, and this was done using a Kruskal-Wallis test of significance (because of non-normal data (Fig. S3)). Analyses were implemented in R3.0.0 (R core team, 2013).

Results

Median thermal preference was significantly different in at least one acclimation group when CTmin values were excluded. This was after acclimation to 0°C where thermal preference was 4.8°C when CTmin values were included, and significantly higher at 9.4°C when values less than 0.2°C were excluded (Wilcoxon rank-sum test: W = 264.5, p = 0.0284; Fig. 1).

Overall, median thermal preference was lower when values within the CTmin range were included (Fig. 1) and since this may have resulted from caterpillars being trapped at their CTmin temperatures (Dillon *et al.* 2012), thermal preference values excluding temperatures less than 0.2°C were used in all further analyses. Furthermore, the distribution of caterpillars on the constant temperature gradient of *c*. 10°C showed that caterpillars were unlikely to favour a particular end of the gradient as a similar number of individuals were found at channels on either end, compared to the distribution of caterpillars on the *c*. 0-30°C gradient where more individuals were found at one end (Fig. S1).

Caterpillars showed a clear preference for low temperatures, and this was particularly true for caterpillars acclimated at 15°C and 5-15°C (Fig. 2). Overall, median thermal preference was *c*. 8°C and, depending on acclimation temperature, these values ranged between 4.6°C and 9.2°C (Table 1). By contrast, median values for T_{opt} were significantly higher and ranged between 20°C and 25°C (Table 1, Fig. 3). In addition, the degree of asymmetry (mean) for locomotor performance at each treatment group was calculated as: 0.31 (field-fresh), 0.36 (0°C), 0.25 (5°C), 0.35 (10°C), 0.38 (15°C), and 0.37 (5-15°C). Since symmetry is reached at zero and values higher than 0.5 indicate highly asymmetrical performance curves (Martin & Huey 2008), the values given here indicate that locomotor performance curves of *P. marioni* are not strongly asymmetric (Fig. 4).

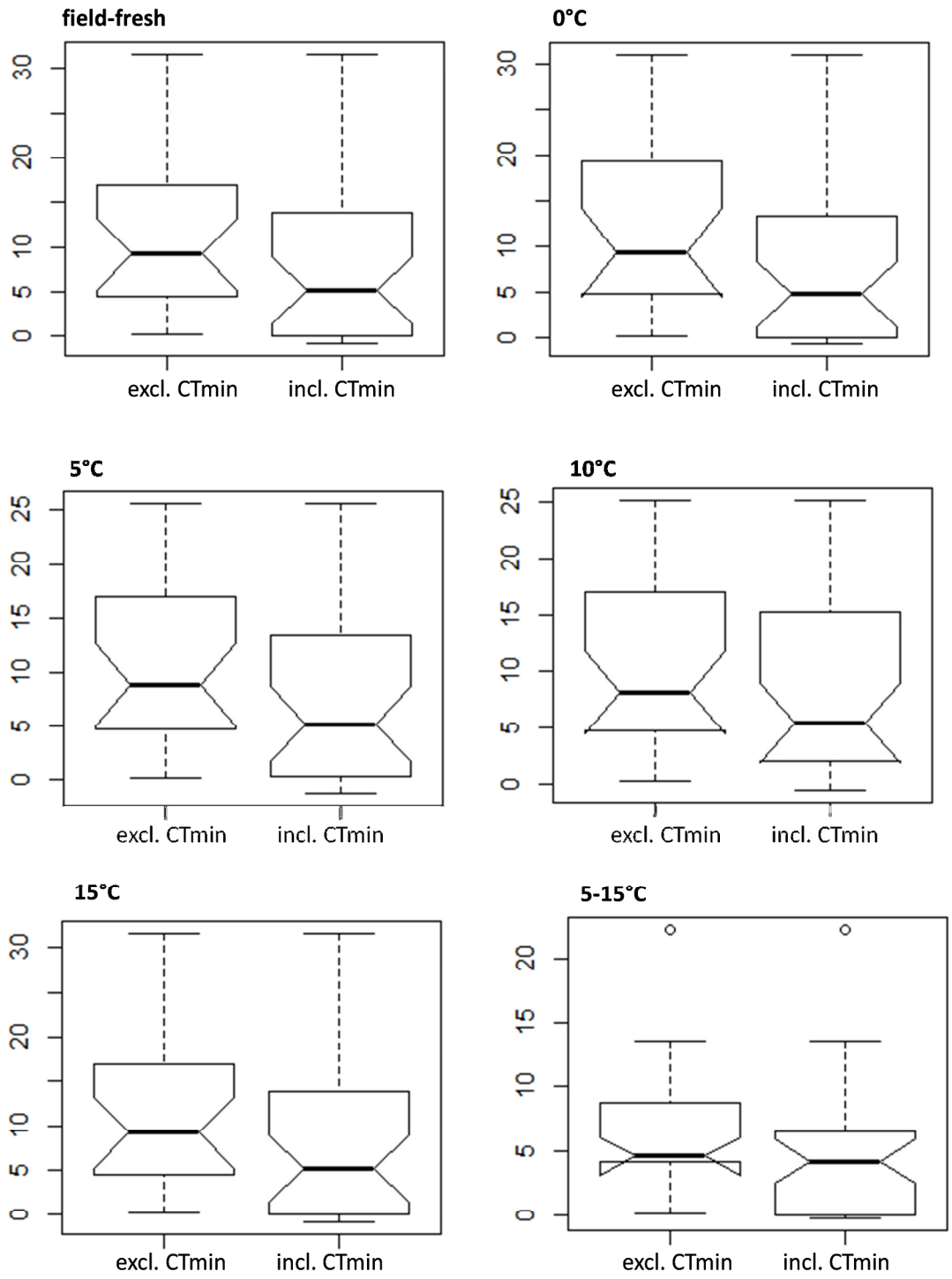


Fig. 1. The difference in the thermal preference (median) of *Pringleophaga marioni* caterpillars when CTmin values are excluded (excl.CTmin) and not (incl.CTmin). This is shown for caterpillars acclimated at 0°C, 5°C, 10°C, 15°C, and 5-15°C, as well as field-fresh individuals. Box plots show the median and interquartile range of thermal preference, and boxes in which notches (i.e. narrowing of the box around the median) overlap are unlikely to have significantly different medians under an appropriate test (Crawley 2007).

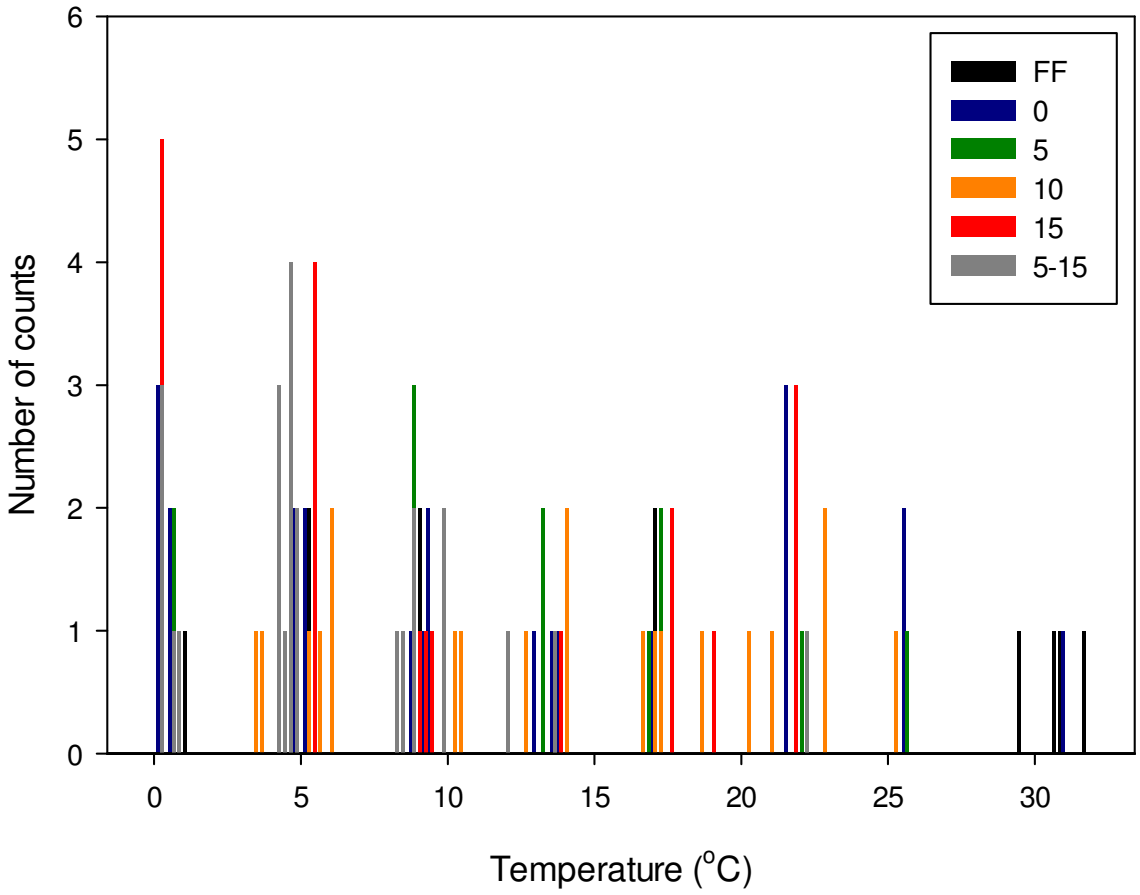


Fig. 2. The thermal preference of *Pringleophaga marioni* caterpillars acclimated at 0°C (blue), 5°C (green), 10°C (orange), 15°C (red), and 5-15°C (grey), and field-fresh individuals (black), as the number of counts on a gradient ranging from *c*. 0-30°C.

Table 1. Median values for thermal preference (T_{pref}) (*c*. 0-30°C gradient), optimum temperature (T_{opt}), the difference between T_{pref} and T_{opt}, and results of the Wilcoxon rank-sum test comparing T_{pref} and T_{opt} are shown for each treatment group.

Group	T_{pref} (°C)	T_{opt} (°C)	Difference (°C)	Wilcoxon rank-sum test
field-fresh acclimation	9.2 (n = 25)	22.5	13.3	W = 583, p < 0.0001
0°C	9.4 (n = 23)	25	15.6	W = 532, p < 0.0001
5°C	8.8 (n = 27)	20	11.2	W = 629, p < 0.0001
10°C	8.1 (n = 28)	22.5	14.4	W = 691, p < 0.0001
15°C	5.2 (n = 29)	25	19.8	W = 776, p < 0.0001
5-15°C	4.6 (n = 24)	20	15.4	W = 657, p < 0.0001

n = sample size

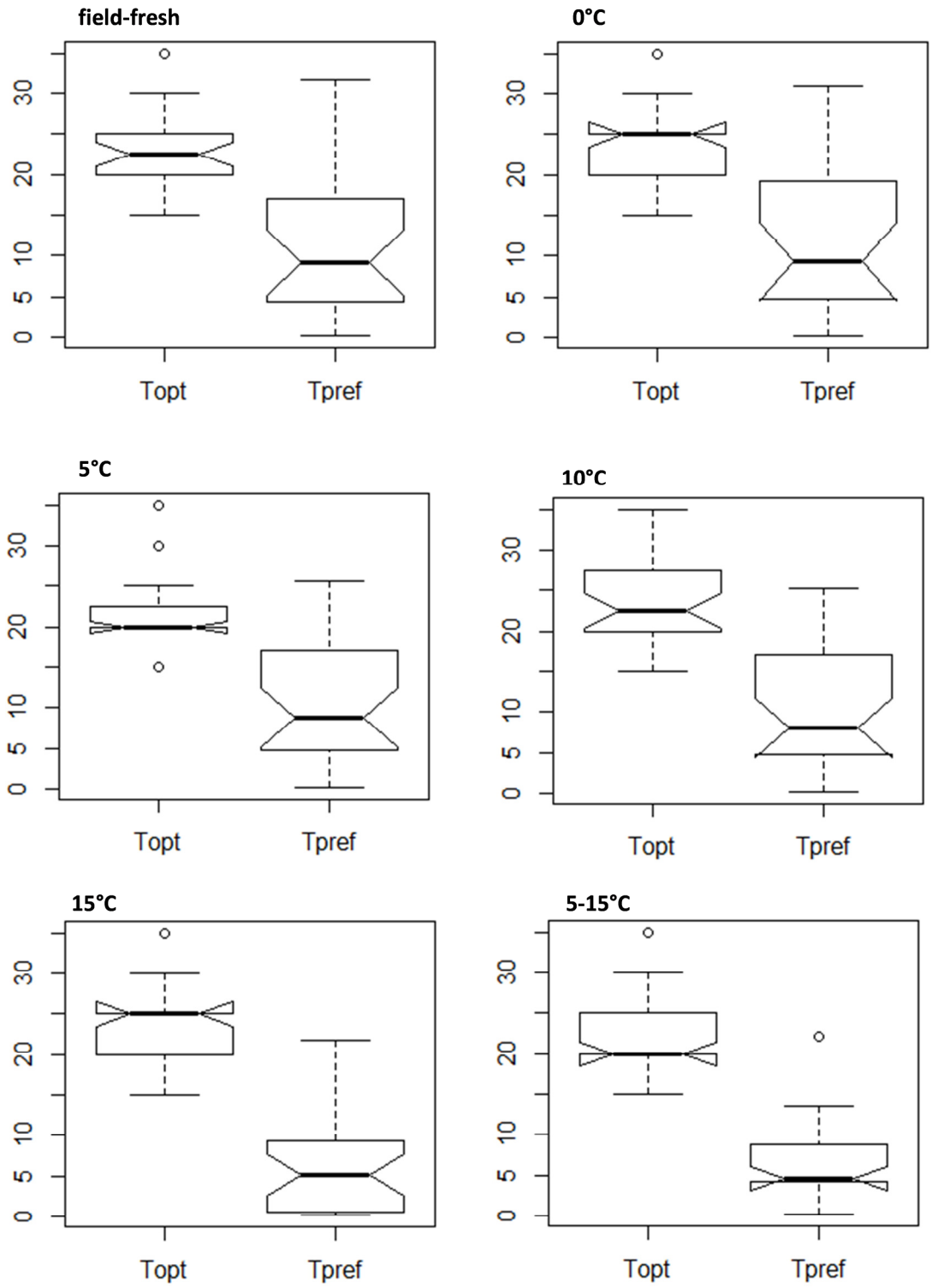


Fig. 3. The difference between the optimum temperature (T_{opt}) and thermal preference (T_{pref}) of *Pringleophaga marioni* caterpillars acclimated at 0°C, 5°C, 10°C, 15°C, and 5-15°C, as well as field-fresh individuals. Box plots show the median and interquartile range for both T_{opt} and T_{pref} and boxes in which notches (i.e. narrowing of the box around the median) do not overlap are likely to have significantly different medians under an appropriate test (Crawley 2007).

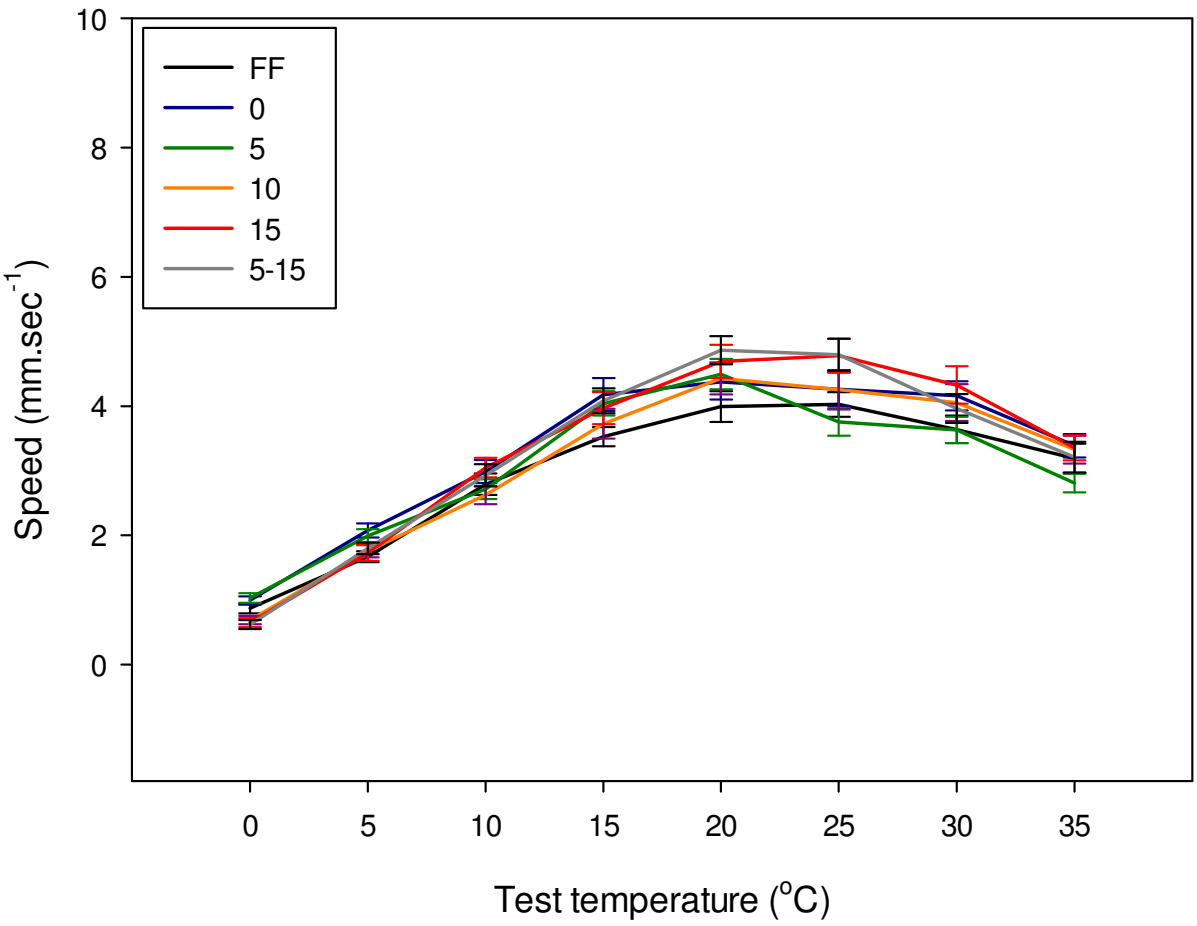


Fig. 4. The locomotor performance of *Pringleophaga marioni* caterpillars, i.e. speed (mm.sec^{-1}) over test temperatures at 0°C to 35°C, at five acclimation treatments: 0°C (blue), 5°C (green), 10°C (purple), 15°C (red) and 5-15°C (grey dashed), and field-fresh (FF) individuals (black) (Mean ± SE).

The mean optimum temperature (T_{opt}), maximum speed (U_{max}) and performance breadth (T_{br}) ranged between 21.4-24.1°C, 4.7-5.4 mm.sec^{-1}, and 16.1-19.8°C respectively (Table 2). Orthogonal polynomial contrast analyses showed that acclimation had neither significant linear nor quadratic effects on any of the performance traits (Table 3). Optimum temperature and maximum speed was not significantly different between the fluctuating temperature regime of 5-15°C and the constant acclimation temperature of 10°C (T_{opt}: $F_{(1,54)} = 0.26$, $p = 0.61$, U_{max}: $F_{(1,54)} = 2.60$, $p = 0.113$), but the performance breadth was

significantly lower at 5-15°C compared to 10°C ($F_{(1,54)} = 5.36$, $p = 0.024$; Table 2). The performance of field fresh individuals also did not differ significantly from those acclimated (T_{opt}: $F_{(5,162)} = 0.87$, $p = 0.503$; U_{max}: $F_{(5,162)} = 1.56$, $p = 0.174$; T_{br}: $F_{(5,162)} = 2.21$, $p = 0.056$). Acclimation also had no significant influence on thermal preference (X^2 (5) = 10.925, p = 0.053).

Table 2. Summary statistics showing means and standard errors for the performance traits: optimum temperature (T_{opt}), maximum speed (U_{max}), and performance breadth (T_{br}).

	T_{opt} (°C)		U_{max} (mm.sec^{-1})		T_{br} (°C)	
	Mean	s.e.	Mean	s.e.	Mean	s.e.
field-fresh	23.8	1.14	4.7	0.18	18.7	0.99
acclimation						
0°C	23.2	1.04	5	0.24	19.8	0.78
5°C	21.4	1.09	4.8	0.20	18.6	0.89
10°C	23.6	1.15	4.9	0.25	18.9	0.92
15℃	24.1	0.86	5.2	0.26	17.8	0.71
5-15℃	22.9	0.79	5.4	0.22	16.1	0.77

s.e. = standard error

Table 3. Outcome of the orthogonal polynomial contrasts on the effects of acclimation on the optimum temperature (T_{opt}), maximum speed (U_{max}), and the performance breadth (T_{br}). In each case, the main effects of acclimation, as well as the orthogonal polynomial contrasts (i.e. linear and quadratic), together with the sign and value of their estimates are shown.

$\boldsymbol{T_{opt}}$					
Source	d.f.	SS	MS	*F*	*P*
acc	3	113	37.72	1.25	0.294
Contrast					
acc linear	1	33	32.54	1.08	0.301
acc quadratic	1	38	37.72	1.25	0.265
Parameter		Estimate	s.e.	*t*	*P*
acc linear		1.08	1.04	1.04	0.301
acc quadratic		1.16	1.04	1.12	0.265
$\boldsymbol{U_{max}}$					
Source	d.f.	SS	MS	*F*	*P*
acc	3	2.63	0.88	0.55	0.647
Contrast					
acc linear	1	0.89	0.89	0.56	0.455
acc quadratic	1	1.64	1.64	1.03	0.312
Parameter		Estimate	s.e.	*t*	*P*
acc linear		0.18	0.24	0.75	0.455
acc quadratic		0.24	0.24	1.02	0.312
$\boldsymbol{T_{br}}$					
Source	d.f.	SS	MS	*F*	*P*
acc	3	58	19.34	1.01	0.391
Contrast					
acc linear	1	24.6	45.57	2.38	0.126
acc quadratic	1	0.3	0.26	0.01	0.908
Parameter		Estimate	s.e.	*t*	*P*
acc linear		-1.28	0.83	-1.54	0.126
acc quadratic		0.10	0.83	0.12	0.908

acc = acclimation temperature
SS = sums of squares; MS = mean squares; s.e. = standard error

Discussion

Wide recognition exists for the coadaptation of preferred and optimal temperatures. A common assumption is that organisms will prefer temperatures at which performance is optimal. However, mismatches do occur, and there are several hypotheses that may explain this departure from coadaptation. The 'immobility hypothesis', preference will be low if organisms are trapped at the lower end of laboratory gradients (Dillon *et al.* 2012). "Suboptimal is optimal', performance decreases rapidly once optimum temperatures are reached and preference should be for lower temperatures (Martin & Huey 2008). The 'thermodynamic effects hypothesis', in the presence of a thermodynamic effect (i.e. poorer performance at low temperatures because biochemical reactions proceed more slowly), natural selection should favour a thermal optimum that exceeds mean body temperature (Asbury & Angilletta 2010). "Trait variation', if optimum temperatures vary among physiological processes, then no single thermal preference will optimize all systems (Huey & Stevenson 1979). The primary goal of this study therefore was to examine the relationship between thermal preference (T_{pref}) and the thermal optimum (T_{opt}) for locomotion of sub-Antarctic *Pringleophaga marioni* caterpillars in light of these hypotheses.

First, to gain an accurate measure of thermal preference, I accounted for the possibility that caterpillars may have been trapped at the cool end of the thermal gradient by excluding all individuals that preferred temperatures within the CTmin range (-1.6°C to 0.1°C (Klok & Chown 1997)) of *P. marioni*. This meant that the 'immobility hypothesis' (Anderson *et al.* 2007; Dillon *et al.* 2012) could be rejected. Preferred temperatures of *P. marioni* (i.e. between 4.6°C and 9.2°C) were lower than optimum temperatures (i.e. between 20°C and 25°C) and the difference between these two traits were large and ranged between 11.2°C and 19.8°C (Table 1; Fig. 3). Large differences (*c.* 8°C) between T_{opt} and T_{pref} have been found in other species, such as in insects: 11°C in *Pterohelaeus darlingensis* beetles (Allsopp *et al.* 1980; Allsopp 1981), in marine invertebrates: 17°C in intertidal snails (*Clorostoma funebralis*) (Tepler *et al.* 2011); and in reptiles: 8°C in lizards (*Hemidactylus turcicus*) (Huey *et al.* 1989; Angilletta *et al.* 1999). Thus, caterpillars of *P. marioni* are part of an extensive group of species showing such large differences. These are much larger than the difference of 1.8°C predicted by the model of Martin & Huey (2008).

Moreover, there was little asymmetry in the performance curves, suggesting that the 'suboptimal is optimal' hypothesis (which assumes asymmetric performance curves) could also be rejected as a possible explanation for the large mismatch between T_{opt} and T_{pref}. In *P. marioni* caterpillars, the mean T_{opt} of *c.* 23°C for locomotor performance is low in comparison

to the upper limiting temperature of *c.* 35°C (Fig. 4), thus accounting for the low asymmetry observed. Locomotion is generally less sensitive than other physiological traits (Angilletta *et al.* 2002a; Martin & Huey 2008), and perhaps a higher degree of asymmetry would be observed in traits such as digestion which may have thermal optimum temperatures that lie relatively close to the upper limiting temperature.

Selection driven by a thermodynamic effect could explain large differences between preferred temperature and the thermal optimum (Asbury & Angilletta 2010). However, in this study, acclimation to high temperatures (e.g. 15°C) did not result in maximal performance (Table 3). I therefore also rejected the 'thermodynamic effect hypothesis' (Asbury & Angilletta 2010). Acclimation had no effect on either locomotor performance (Table 3), or thermal preference (Table 1), indicating a lack of phenotypic plasticity for this species. A lack of plasticity might be expected in some circumstances at Marion Island (Klok & Chown 1998; Deere & Chown 2006; Deere *et al.* 2006; Marais *et al.* 2009), and this may be as a result of environmental unpredictability, i.e. the risk of misleading environmental cues resulting in inappropriate responses (Chown & Terblanche 2007). Temperatures on Marion Island are highly unpredictable from day to day (Deere *et al.* 2006), meaning that organisms may receive environmental cues that are likely to be poor predictors of future environmental conditions, thus, leading to fixed rather than plastic phenotypes (Tufto 2000; Deere & Chown 2006; Alfaro *et al.* 2013). Consequently, invertebrate species occurring on Marion Island have also shown no response to acclimation for various traits. For example, acclimation had no effect on CTmin temperatures in lepidopteran larvae (Klok & Chown 1998), lower lethal limits (Deere *et al.* 2006) and locomotor performance in mites (Deere & Chown 2006), thermal tolerance in indigenous springtails (Slabber *et al.* 2007), and also survival of cold shock in kelp fly larvae and adults (Marais *et al.* 2009).

When measuring ectotherm performance, physiologists often examine both variable and stable environments as the former may represent more ecologically-realistic conditions (e.g. Folguera *et al*. 2009; Kingsolver *et al.* 2009; Lyons *et al.* 2013). Caterpillars acclimated to the fluctuating temperature of 5-15°C had a significantly lower performance breadth compared to caterpillars exposed to the constant temperature of 10°C (i.e. closest comparable mean temperature). The performance breadth is the one measure of performance that is expected to change significantly in fluctuating as opposed to constant temperatures (Huey & Slatkin 1976; Huey & Stevenson 1979; Huey & Kingsolver 1993; Huey *et al.* 1999). Although 15°C lies within the microhabitat temperature range of *P. marioni* (Chown & Crafford 1992), survival at this temperature is particularly low (Haupt *et al.* 2013). Median

values for thermal preference were also lower at 5-15°C (4.6°C) compared to 10°C (8.1°C) (Table 1). Animals often compensate for exposure to high temperatures by seeking out lower temperatures (e.g. Gvoždík *et al.* 2007; Qu *et al.* 2011; but see Hutchison & Hill 1976 and Hadamová & Gvoždík 2011). Thus, the significantly lower T_{br} after exposure to the fluctuating temperature is more likely a deleterious response to the high temperature extreme of 15°C.

Since neither the 'immobility hypothesis', 'suboptimal is optimal', nor 'thermodynamic effect hypothesis', could explain the large variation in T_{opt} and T_{pref}, I considered the 'trait variation' hypothesis. That is, T_{pref} may align with some other measure of performance that may be more significant for a detritivorous caterpillar. For example, if the optimum temperature for locomotion is higher than that for growth, then animals may choose a high preference temperature only when the ability to move faster is of more immediate importance than the ability to grow quickly (Huey & Stevenson 1979; Anderson *et al.* 2011). Thus, preference may be towards physiological systems that improve fitness (e.g. Miller *et al.* 2009; Coggan *et al.* 2011; Clissold *et al.* 2013). For example, caterpillar survival to pupation is far higher at temperatures lower than 15°C, i.e. at 5°C or 10°C (Haupt *et al.* 2013). Similarly, 10°C is the optimum temperature for caterpillar feeding (Crafford 1990), and low thermal preferences may be linked to nutrient or digestive efficiency of caterpillars (Crafford 1990). Compared to the optimum temperature for locomotion (*c.* 23°C), these temperatures more closely match the preferred temperatures of 4.6°C to 9.2°C. These findings therefore provide support for the 'trait variation' hypothesis which may explain the large mismatch observed between T_{pref} and T_{opt}.

For *P. marioni* caterpillars on Marion Island, locomotion may be important for locating food resources and suitable microhabitats that minimise predation, and these may all have direct consequences for survival. However, the low preferred temperatures compared to the optimum temperature for locomotion found in this study, suggests that high optimum temperatures may not be the most ecologically optimal temperatures for this species. Instead, caterpillars may prefer lower temperatures where survival and assimilation efficiency is maximal, or where costs associated with high temperatures are minimized.

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Supplementary material

Table S1. Thermal preference of *Pringleophaga marioni* caterpillars on a gradient of *c*. 0-15°C (medians of thermal preference are shown for each acclimation temperature).

Acclimation temperature (°C)	Thermal preference (°C)
0°C	6.7
5°C	9.5
10°C	6.6
15°C	7
5-15°C	7.3

Table S2. Results from a Shapiro-Wilk's test for normality and Levene's test for homogeneity of variances for each test conducted on T_{opt}, U_{max} and T_{br}. These were: i) an ANOVA comparing all groups together, i.e. field fresh, 0°C, 5°C, 10°C, 15°C and 5-15°C, ii) orthogonal polynomial contrast analyses, which compared the equally spaced acclimation temperatures of 0°C, 5°C, 10°C and 15°C, and iii) an ANOVA in which the 5-15°C and 10°C acclimation temperatures were compared to examine the effects of fluctuating versus constant acclimation temperatures.

Test	Shapiro-Wilk's	Levene's
All groups		
T_{opt}	W = 0.89, p < 0.0000	F = 1.21, d.f. = 5,162, p = 0.3044
U_{max}	W = 0.99, p = 0.5262	F = 0.64, d.f. = 5,166, p = 0.5664
T_{br}	W = 0.93, p < 0.0000	F = 0.78, d.f. = 5,166, p = 0.5664
0, 5, 10, and 15°C		
T_{opt}	W = 0.89, p < 0.0000	F = 0.96, d.f. = 3,108, p = 0.4127
U_{max}	W = 0.99, p = 0.5262	F = 0.29, d.f. = 3,108, p = 0.8348
T_{br}	W = 0.94, p < 0.0000	F = 0.61, d.f. = 3,108, p = 0.6120
5-15 and 10°C		
T_{opt}	W = 0.89, p < 0.0000	F = 4.33, d.f. = 1,54, p = 0.0423
U_{max}	W = 0.99, p = 0.5262	F = 0.61, d.f. = 1,54, p = 0.4393
T_{br}	W = 0.93, p < 0.0000	F = 1.37, d.f. = 1,54, p = 0.2467

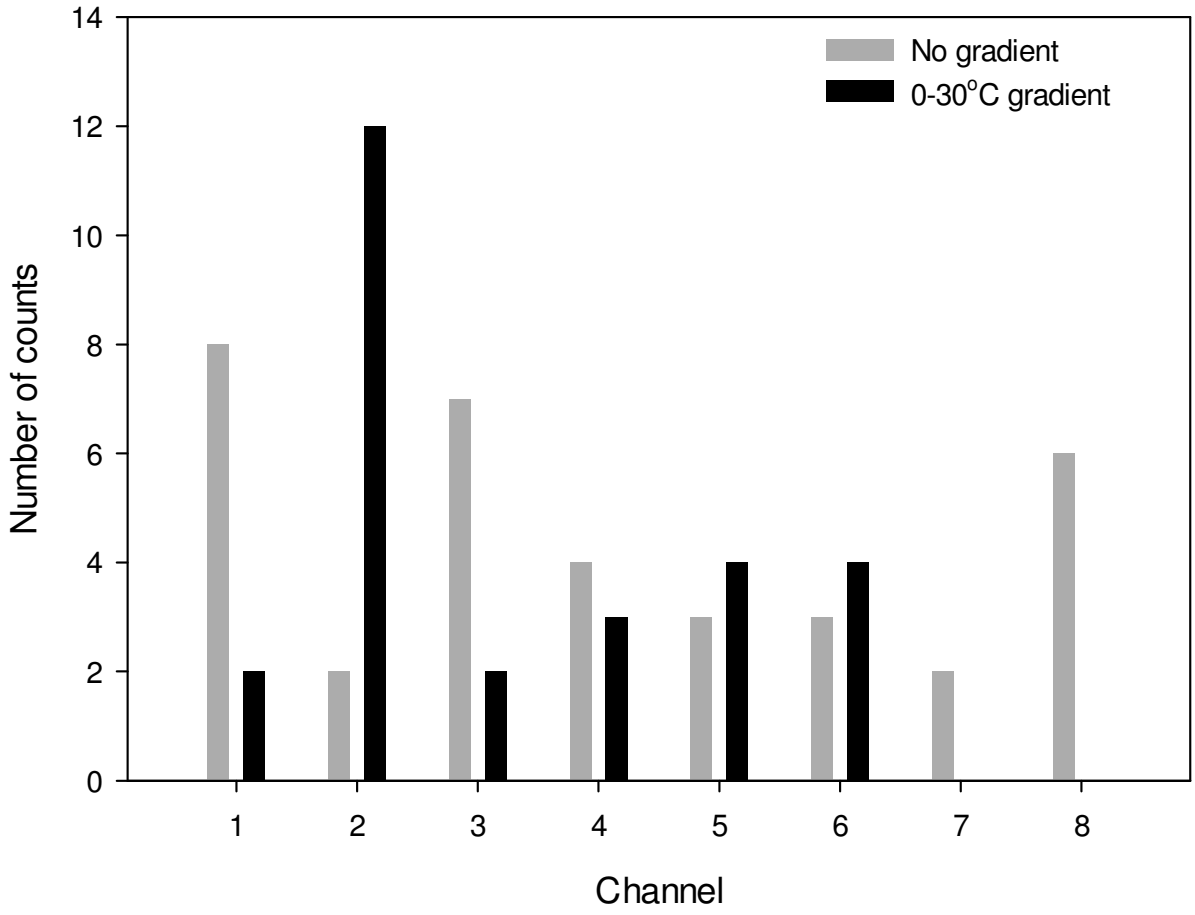


Fig. S1. The distribution of 10°C acclimated individuals on the gradient at a constant temperature of *c.* 10°C (n = 35, grey bars), compared to temperatures ranging from *c*. 0-30°C (n = 28, black bars).

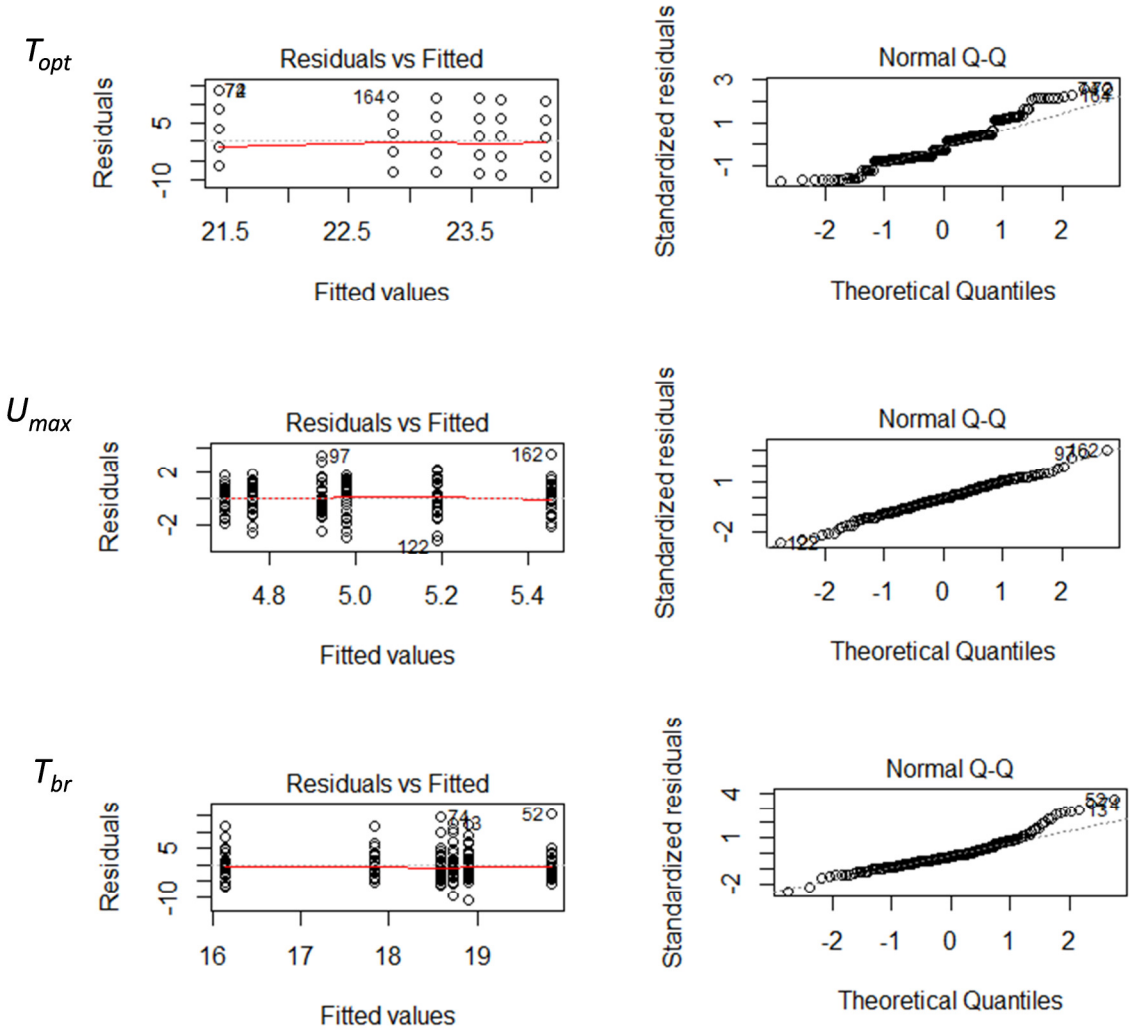


Fig. S2a. Model assumption plots (i.e. normal probability plots and residual versus fitted value plots) to test normality and equal variances for the ANOVA in which performance measures (i.e. T_{opt}, U_{max} and T_{br}) between all groups (i.e. field fresh, 0°C, 5°C, 10°C, 15°C and 5-15°C acclimation temperatures) were compared.

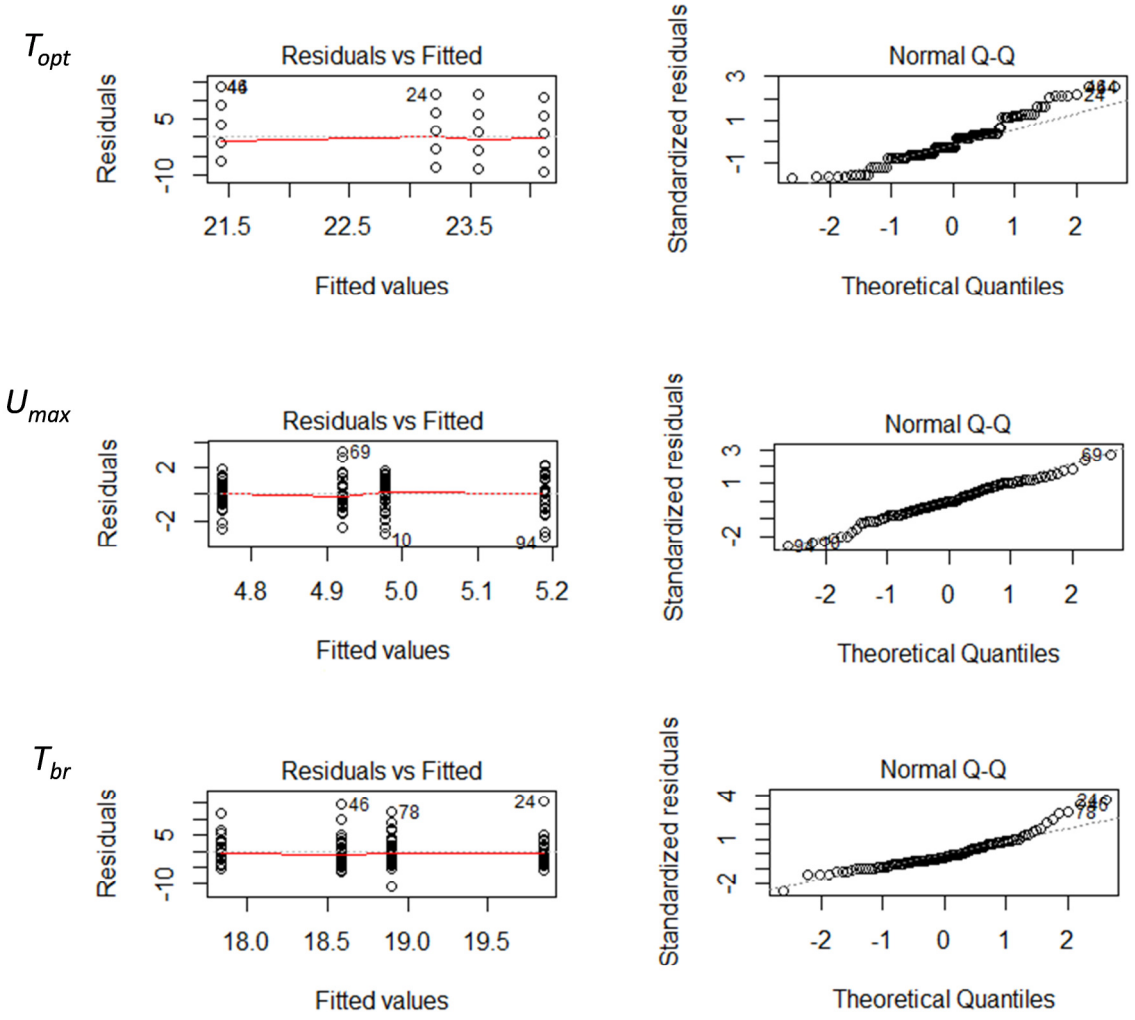


Fig. S2b. Model assumption plots (i.e. normal probability plots and residual versus fitted value plots) to test normality and equal variances for the orthogonal polynomial contrast analyses in which performance measures (i.e. T_{opt}, U_{max} and T_{br}) between the 0°C, 5°C, 10°C and 15°C acclimation temperatures were compared.

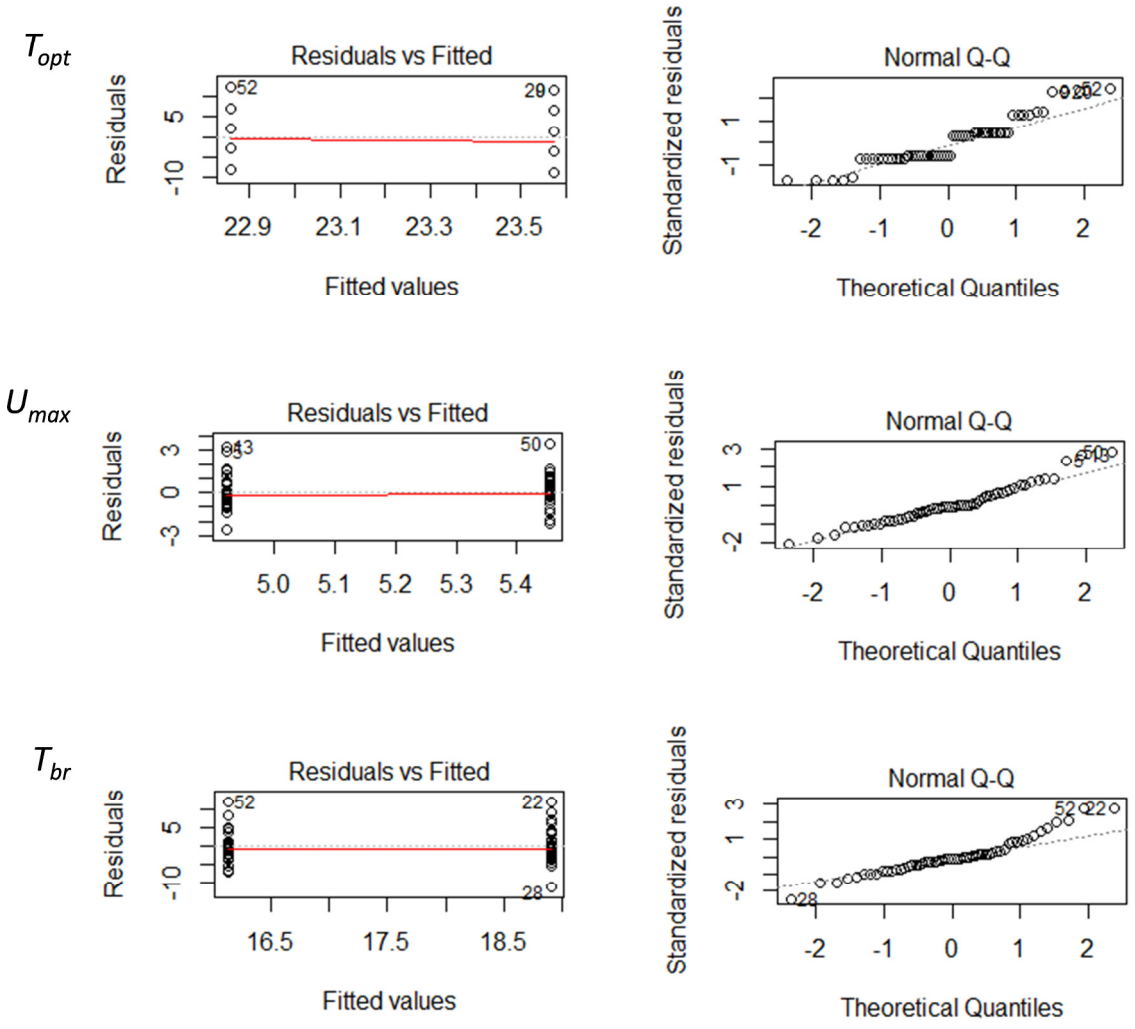
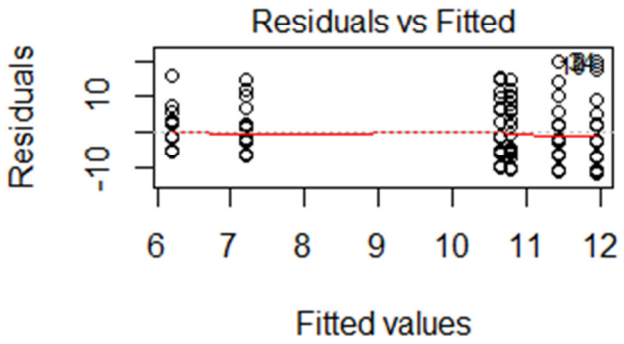


Fig. S2c. Model assumption plots (i.e. normal probability plots and residual versus fitted value plots) to test normality and equal variances for the anova in which performance measures (i.e. T_{opt}, U_{max} and T_{br}) between the fluctuating temperature of 5-15°C and the constant temperature of 10°C were compared.

(a)



(b)

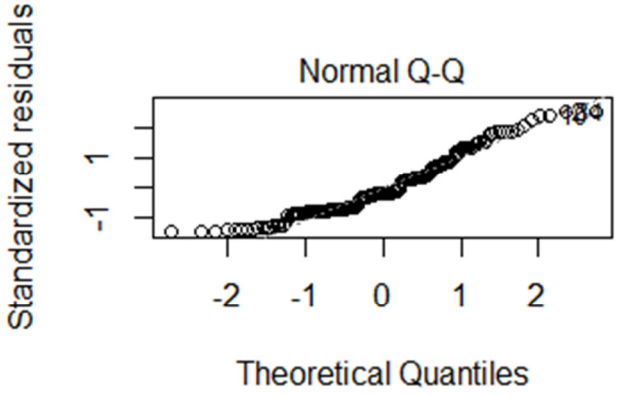
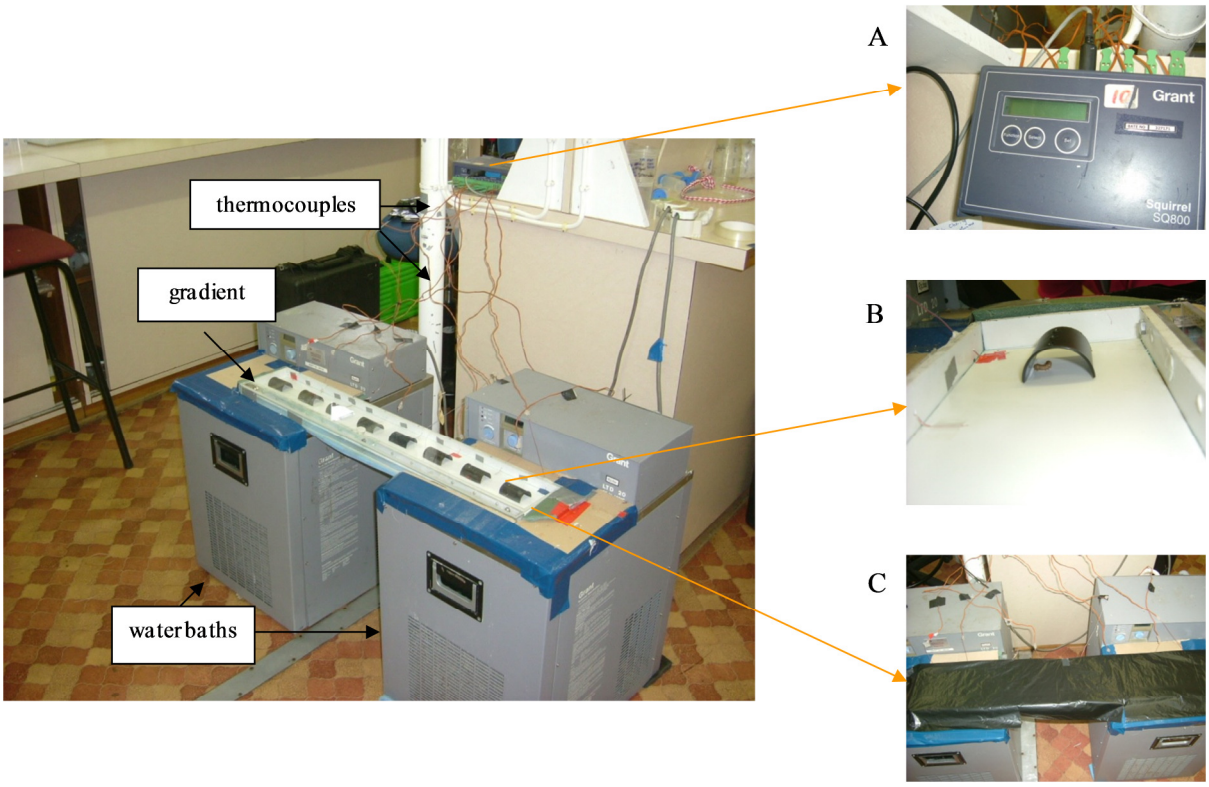


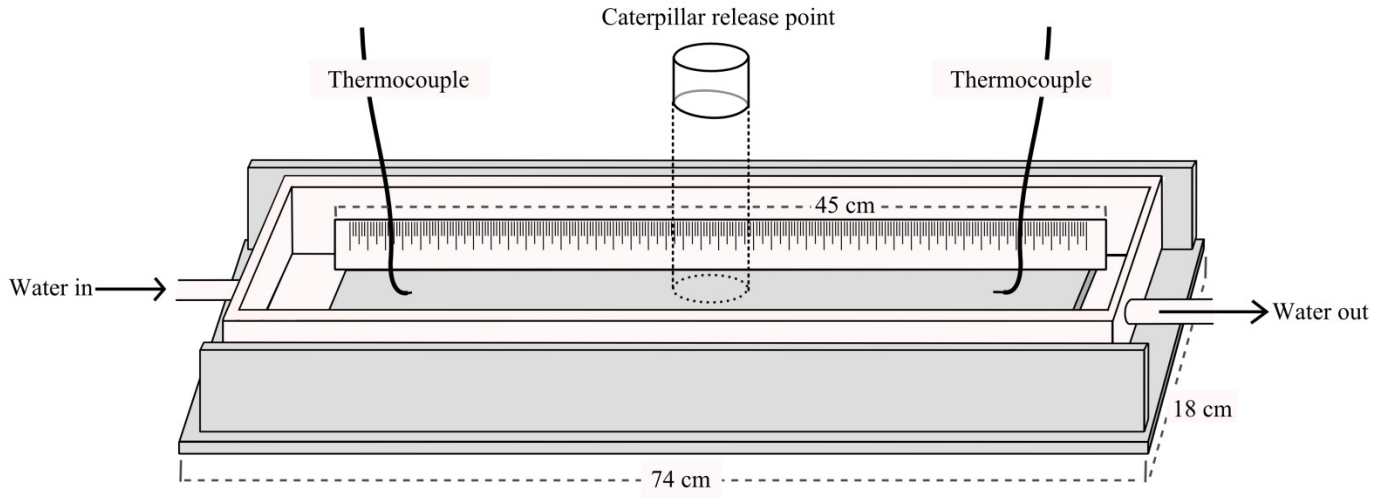
Fig. S3. Residual plots of thermal preference at all acclimation temperatures (0°C, 5°C, 10°C, 15°C and 5-15°C), as well as field fresh individuals on a gradient of *c*. 0-30°C showing (a) the residuals versus fitted values, and (b) a normal probability plot. A Shapiro-Wilk's test indicated that data were not normally distributed ($W = 0.91$, $p < 0.0001$), and a Levene's test showed that variances were not equal ($F = 3.26$, d.f. = 5,150, $p = 0.008$).

Appendices

Appendix A. The experimental set-up of the thermal preference gradient with inserts of the squirrel data logger (A), plastic refuges (B), and the stage covered with a black plastic bag to eliminate light (C).



Appendix B. The walking stage used in locomotor performance trials (details are given in the text).



Chapter 6 – Chemosensory and thermal cue responses in the sub-Antarctic moth *Pringleophaga marioni*: do caterpillars seek out wandering albatross nest proxies?

Female adult moth, *Pringleophaga marioni.*
Photo credit: O. Whitehead

[1] T.M. Haupt, B.J. Sinclair & S.L. Chown (in press). Chemosensory and thermal cue responses in the sub-Antarctic moth *Pringleophaga marioni*: do caterpillars seek out wandering albatross nest proxies? *Polar Biology*

Abstract

On the South Indian Ocean Province Islands of the sub-Antarctic, most nutrients are processed through a detritus-based food web. On Marion Island, larvae of the moth *Pringleophaga marioni* are one of the key decomposers. Abundance of these caterpillars is generally higher in newly abandoned wandering albatross (*Diomedea exulans*) nests than other habitats, and this observation has been explained by hypotheses regarding the thermal and nutrient advantages of nests. These hypotheses require a mechanism for increasing the abundance of caterpillars, since nests are an ephemeral resource, and here I determine whether caterpillars respond to chemosensory and thermal cues using a laboratory choice chamber approach. Caterpillars show no significant preference for newly abandoned nest material over no other choice, old nest material, and the common mire moss *Sanionia uncinata*. Caterpillars that are acclimated to warm (15°C) conditions do prefer lower (5°C) to higher (15°C) temperatures, perhaps reflecting negative effects of prolonged exposure to warm temperatures on growth. Caterpillars also show significant avoidance of conspecifics, possibly because of incidental cannibalism previously reported in this species. Thus, I find no empirical support for nest-finding ability in caterpillars based on chemosensory or thermal cues. It is possible that adult females or very early instar caterpillars show such ability, or high caterpillar density and biomass in nests are an incidental consequence of better conditions in the nests or deposition by the birds during nest construction.

Keywords: Antarctic - ecosystem engineering - habitat choice – chemosensory - thermal preference

Introduction

Sub-Antarctic Island terrestrial ecosystems are depauperate in herbivores and dominated by a detritivore food chain. The predominant invertebrate decomposers are oligochaete worms, dipteran larvae, and coleopteran adults and larvae, and on the South Indian Ocean Province (SIOP) Islands, caterpillars of the flightless moth genus *Pringleophaga* (Holdgate 1977; Tréhen *et al.* 1985; Crafford *et al*. 1986; Smith and Steenkamp 1992; Chown and Convey 2007). Understanding the factors that determine the abundance and distribution of these key decomposers is important for several reasons. First, they contribute substantially to terrestrial food web structure and dynamics, about which little is known (Burger 1985; review in Hogg *et al.* 2006; Bokhorst *et al.* 2007; Smith 2008). Understanding distribution and abundance is a key first step in elucidating food web structure (Cohen *et al*. 2003). Second, decomposition processes on the SIOP Islands are being influenced by climate change through both direct effects on the decomposers (e.g. Smith and Steenkamp 1990; Chown *et al*. 2007) and via interactions with introduced predators that consume the major decomposers (Bergstrom and Chown 1999; Le Roux *et al.* 2002; Smith 2002). Third, the introduction of non-indigenous species that have become invasive are either having, or are suspected of having, both direct (Hänel & Chown 1998; Lebouvier *et al*. 2011) and indirect effects (Terauds *et al*. 2011) on decomposition.

The high biomass of flightless moth caterpillars (*Pringleophaga marioni* or related species on other islands) in or near wandering albatross nests (*Diomedea exulans*) is well-known (Joly *et al*. 1987; Crafford *et al*. 1986). Previous investigations suggested that these high densities are a consequence of high nutrient (mostly N) availability around and in nests (Smith 1976; 1978; 1979; Joly *et al*. 1987). A range of invertebrates on the islands respond positively in abundance and richness to high nutrient habitats (e.g. Burger 1978; Gabriel *et al*. 2001). This 'nutrient hypothesis' implies that fitness benefits to *P. marioni* derive from high nutrient availability.

Subsequently, it has been suggested nutrients might not be responsible for high caterpillar densities and biomasses in nests on Marion Island, because nutrient concentrations in nests were either similar to or lower than that of other island habitats (Sinclair & Chown 2006). Rather, a role for temperature was proposed given that nests occupied by the birds were approximately 5°C warmer than abandoned nests and surrounding soils. Thus, wandering albatrosses serve as thermal ecosystem engineers to caterpillars by providing a warm microhabitat in which caterpillar growth, survival and thus fitness are assumed to be improved.

Both the 'nutrient hypothesis', and the new 'thermal ecosystem engineering' hypothesis, are ultimate explanations for higher caterpillar biomass in nests. These hypotheses suggest that because caterpillar fitness are elevated in nests, either ovipositing female moths or caterpillars might preferentially seek out nests, so explaining the low variation in biomass among nests compared with that among sites in the islands major lowland habitats. Nest-finding ability is a proximate mechanism through which the fitness benefits come to be realized, especially because the nests are ephemeral (lasting a year, given the biennial breeding cycle of the birds, but may be used again after an unoccupied period – Ryan & Bester 2008), and the duration of the larval stage of *P. marioni* is also less than a year (Haupt *et al*. 2013). By contrast, another proximate explanation is that higher biomass/density is an incidental outcome of higher growth rates and survival in nests following a distribution of eggs by ovipositing females that is no different to that found in other habitats. Thus, no selection for nest-finding ability has taken place. This 'incidental hypothesis' has a further form. Because wandering albatross pairs build their nests from the surrounding vegetation (Ryan & Bester 2008), which have high caterpillar abundances (Joly *et al*. 1987), caterpillars or eggs might be inadvertently incorporated into nests during nest building. Caterpillars would then continue to have higher survival and growth rates in the nests than elsewhere as suggested by Sinclair and Chown (2006). Another hypothesis is that of 'differential mortality' – i.e. caterpillars are distributed evenly in both nests and surrounding habitats but nests prevent or minimise predation by introduced house mice, *Mus musculus* (Chown *et al.* 2002; Smith *et al.* 2002). Thus, four non-exclusive potential mechanisms are identified which may explain the high caterpillar abundance in nests: (i) nest-finding by either caterpillars or ovipositing female moths; (ii) increased growth and survival once in the nest mediated by elevated temperatures or nutrient input; (iii) incidental incorporation; and (iv) differential mortality. In this study, part of the first hypothesis was addressed.

Selection for nest-finding is in keeping with what has been found in other insect species. Oviposition sites which maximize offspring performance are often carefully selected (Thompson 1988; Nylin & Janz 1993; Huk & Kühne 1999; Scheirs *et al*. 2000; Ganehiarachchi *et al*. 2013), and larvae of other Lepidoptera preferentially move to advantageous microhabitats (Alonso 1997; Soler *et al*. 2012). The use of visual (e.g. Stefanescu *et al.* 2006; Stenberg & Ericson 2007) and chemosensory cues (e.g. Bruce *et al*. 2005; Hamilton *et al.* 2011; Sim *et al*. 2012; Anderson *et al*. 2013) are common in identifying host plants, habitats, or oviposition sites. Similarly, insects sense and respond to temperature

variation and use thermal cues in locating habitats or food resources (e.g. Lazzari & Núñez 1989; Alonso 1997; Flores & Lazzari 1996; Chown & Terblanche 2007; Kleinedam *et al*. 2007; McClure *et al.* 2011; Clissold *et al.* 2013).

Here I therefore tested explicitly the hypothesis that caterpillars are able to actively choose wandering albatross nests as a preferred habitat. I refer specifically to occupied nests, but using newly abandoned nests (from the current season, abandoned within the past six weeks) as a proxy for them given constraints on sampling occupied nests (see Sinclair & Chown 2006). Given that other insects may use chemosensory or thermal cues for site or host selection, I predicted that caterpillars should prefer nest over non-nest material, new nest material rather than material from long abandoned (old) nests, and temperatures more typical of nests than the surrounding areas. Because caterpillars of some other lepidopteran species are known to derive fitness benefits from aggregation and respond to conspecific cues (Klok & Chown 1999; Colasurdo & Despland 2005; Wertheim *et al*. 2005; Ronnås *et al*. 2011), I also examined the hypothesis that caterpillars respond to conspecifics to maximize fitness benefits.

Materials and methods

Study system

Pringleophaga marioni Viette is a flightless tineid moth with a life cycle of approximately one year at habitat temperatures typical of the lowlands of Marion Island (*c*. 5-7°C, Deere *et al*. 2006), and which feeds predominantly on detritus (Crafford *et al*. 1986; Haupt *et al*. 2013). On Marion Island, *P. marioni* is found in a large variety of habitats, most notably in the mire and biotically-influenced communities along the coast, but also in high elevation sites of up to 800 m above sea level (Crafford *et al*. 1986). Among-site variation in abundance is high, but habitats influenced by animals (e.g. *Poa cookii* tussock grasslands surrounding penguin colonies) tend to have the highest abundances, with newly-abandoned wandering albatross nests being most consistent in this respect (e.g. Burger 1978; Chown *et al*. 2002; Sinclair & Chown 2006).

Wandering albatrosses nest on the SIOP Islands, and build large nests (*c*. 20 cm high and 100 cm diameter (Fig. 1)) composed of plant material and peat collected from their surroundings (Joly *et al.* 1987; Ryan & Bester 2008). For this study, nest material was collected from both ‘old’ and ‘newly-abandoned’ nests, and all caterpillars removed via hand sorting. ‘Old’ nests are those that have been abandoned by the birds for more than one year (either after chick fledging or nest failure) (Fig. 1c). Old nests are typically flat and

overgrown with vegetation, whereas newly abandoned nests have no or very little vegetation on them. Newly-abandoned nests (hereafter 'new nests') can also be identified by tufts of vegetation scattered around the nest, an indication of recent nest building activities, and sometimes also by remnants of egg shell and feather down (Sinclair & Chown 2006) (Fig. 1 b).

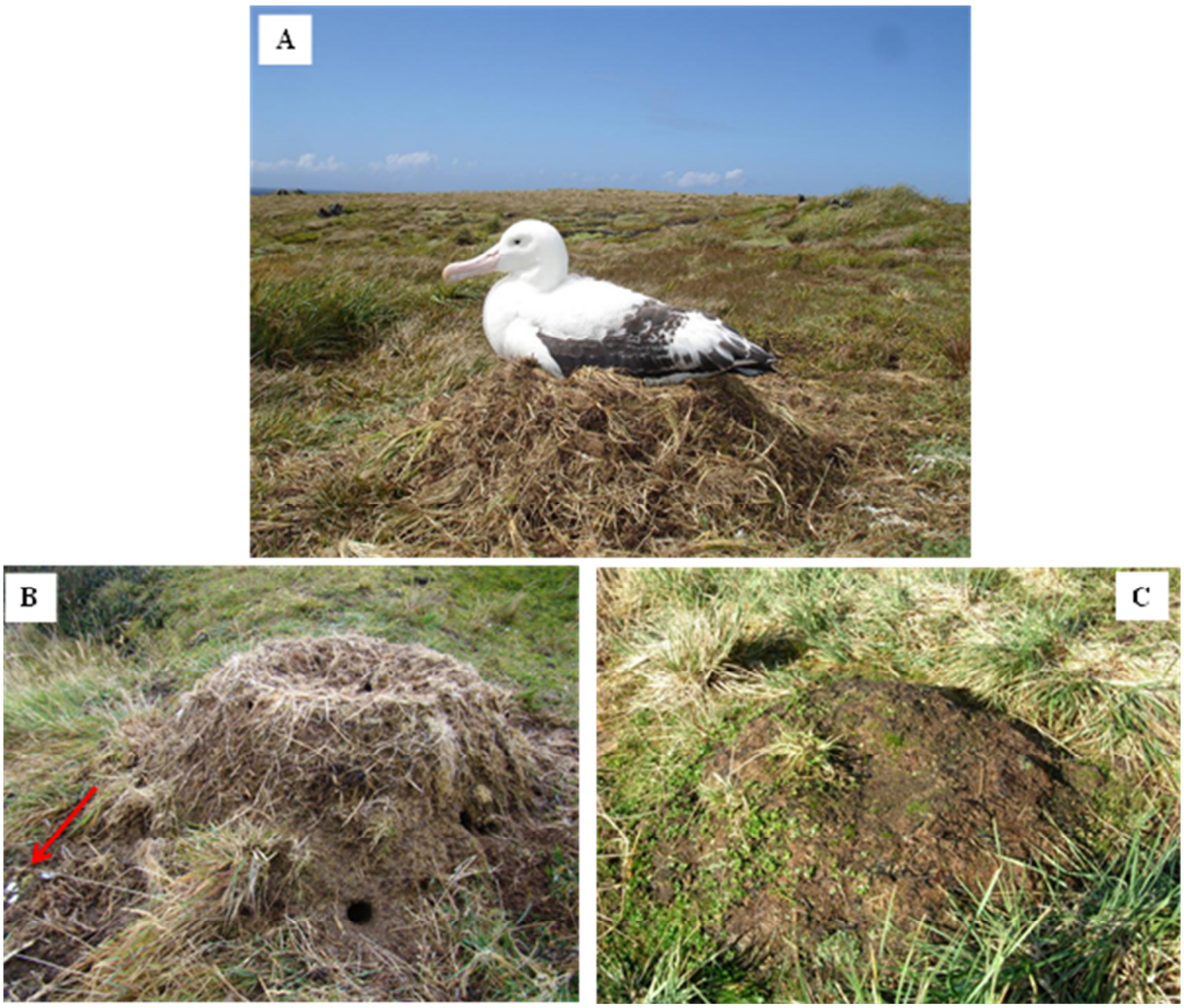


Fig. 1. (A) Nesting wandering albatross. (B) A newly abandoned nest where remnants of eggshells are apparent on the side of the nest (arrow) indicating fairly recent nest occupancy. A mouse burrow is also visible. (C) An old nest trampled flat and covered with vegetation.

Choice experiments

Caterpillars (typically in the 70 to 100 mg size range, therefore not in the first instar, as these stages are scarce during the period of fieldwork (Haupt *et al.* 2013)) were collected by hand from abandoned wandering albatross nests located in the vicinity of the research station on

Marion Island and returned to the laboratory within six hours of collection. In the laboratory, individuals were held separately in Petri dishes filled with homogenized albatross nest material (mixed by hand from material from several field-collected nests to prevent any systematic biases in composition or moisture), which served as both refuge and food (see Haupt *et al*. 2013), in climate chambers (MIR 154, Sanyo, Osaka, Japan, accurate to ± 0.5°C, temperatures given below) with a photoperiod of 12L:12D (set according to the seasonal schedule of Marion Island). Keeping individuals separate was necessary to avoid cannibalism (French & Smith 1983).

The custom-made choice apparatus was built from stainless steel and plexiglass and fitted with a lid. It comprised four chambers in an X-design, in line with similar choice experiment designs used elsewhere (see e.g. Raffa *et al*. 2002; Soler *et al*. 2012), with independent temperature control for the left and right hand sets of chambers (Fig. 2). Temperature control was achieved via a water/propylene glycol mixture pumped through the base of the chambers from each of two Grant LTC 12 water baths (Grant Instruments, Cambridge, UK). Type-T thermocouples were affixed to the floor of each chamber and temperatures read from a CHY 507 (Firemate Co., Taiwan) thermocouple thermometer, so ensuring that the chamber temperatures were within ± 1°C of the pre-selected temperature. The choice chamber was kept completely dark by covering it with aluminium foil. At the start of each choice trial, individuals were placed in the centre of the chamber and held under a plastic container for four minutes to equilibrate. After four minutes the caterpillar was released remotely, and left to move for ten minutes after which the chamber in which it was found was recorded. Preliminary trials showed that caterpillars (*c.* 2cm in length) could move to more than one chamber within a 10 minute period (with locomotion speed experiments demonstrating an average distance moved of 30 cm in 10 minutes at 0°C and 240 cm at 15°C, Chapter 5), but not directly from one chamber to the next. After each trial, the entire apparatus was cleaned with 100% ethanol.

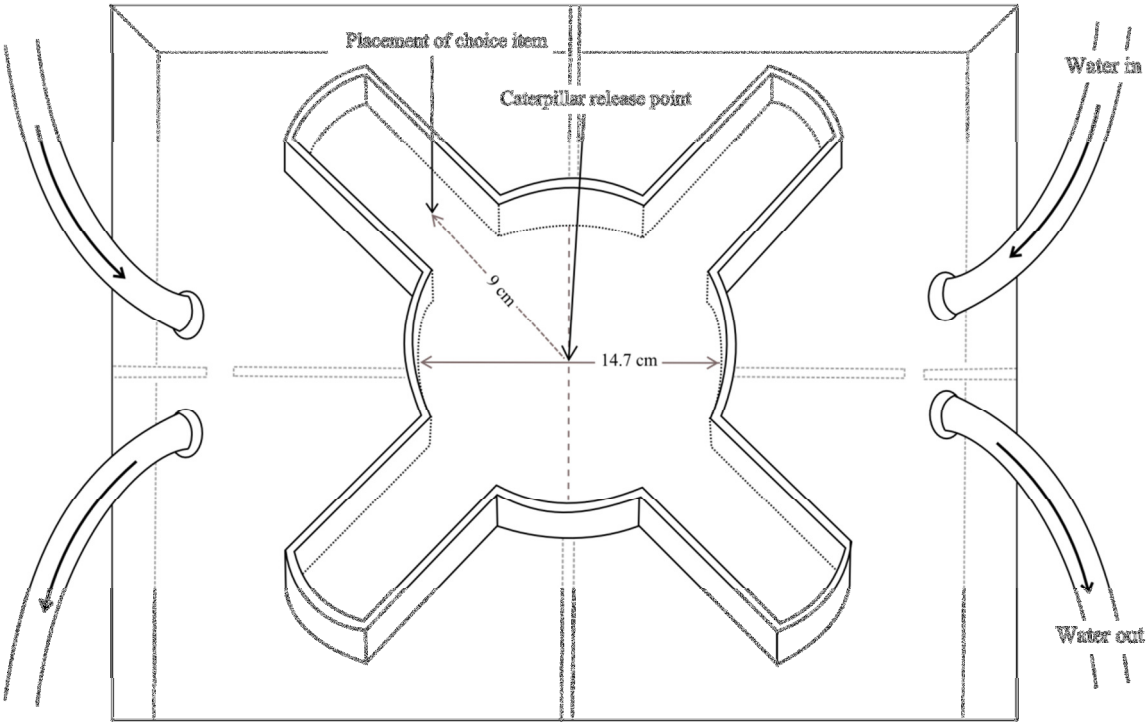


Fig. 2. Schematic diagram of the choice chamber design and temperature control for trials of preference in *P. marioni* caterpillars. Water flowed in and out of pvc piping attached to water baths (not shown here) which enabled independent temperature control of the left and right hand sides of the apparatus. The position at which an individual caterpillar was released and the distance from this point to where nest material, *Sanionia uncinata*, or caterpillar cages were placed, are also shown.

Six choice experiments were undertaken:

1. A choice between new nest material and nothing to determine if caterpillars select nest material.

2. A choice between new nest material and *Sanionia uncinata*, a moss typical of mire communities (Gremmen 1981), and in which *P. marioni* caterpillars are commonly found (Burger 1978).

3. A choice between new and old nest material to determine if caterpillars use chemosensory cues to distinguish between the two types of nests.

4. A choice between chambers in which other *P. marioni* caterpillars were either present or not, to determine if caterpillars are attracted or deterred by other caterpillars. Here, caterpillars in the choice chambers were held in small mesh cages, and the chambers without caterpillars contained mesh cages only to serve as procedural controls.

For these four experiments, caterpillars were initially held in the incubators at 5°C (± 0.5°C) for no longer than three days. The trials were then run at 15°C (± 1°C) in the chambers, with two chambers containing one of the choices and two chambers the other, selected at random for each individual trial. A temperature of 15°C was selected because it is *c*. 5°C below the optimal locomotion speed for caterpillars (Chapter 5), and well above the critical thermal minimum (Klok and Chown 1997) so ensuring that choice would not be compromised by restricted movement ability (Dillon *et al*. 2012).

5. Thermal choices in which caterpillars were presented with warm and cool chambers to determine if they respond to thermal cues. Temperatures of the chambers were first set at 0°C vs. 10°C, and then at 5°C vs. 15°C in a design identical to the previous choice experiments. These temperatures essentially reflect the range of optimal and stressful temperatures for caterpillars (Sinclair & Chown 2005; Haupt *et al*. 2013) despite much broader short-term thermal limits (Klok & Chown 1997). Caterpillars were acclimated for seven days at 0°C (choice of 0°C vs. 10°C) or 5°C (choice of 5°C vs. 15°C). These acclimation temperatures were used to reflect winter and summer conditions on the island (for climate data see Le Roux & McGeoch 2008), and the timing of acclimation period was based on previous trials showing acclimation responses within a week for this species (Sinclair & Chown 2003) and for insects generally (Weldon *et al*. 2011).

6. A choice between old and new nest material at temperatures either close to the optimum movement temperature (15°C), or much lower and at which growth is relatively slow (5°C) (Haupt *et al*. 2013). This experiment meant that each of the four chambers represented a unique choice: (1) new nest material at 5°C; (2) old nest material at 5°C; (3) new nest material at 15°C; and (4) old nest material at 15°C. This experiment examined whether caterpillars respond to thermal and chemosensory cues simultaneously. In advance of this experiment, caterpillars were held at 5°C for no longer than three days.

Nest material used in the experiments was homogenized and kept in climate chambers (MIR 154, Sanyo, Osaka, Japan, accurate to ± 0.5°C) at 5°C (choices 1-3), or in the case of experiment 6, at 5°C and 15°C (i.e. for 5°C and 15°C chambers respectively) for no longer than 10 days. For each trial, similar-sized portions (*c*. 50 ml) of nest material or vegetation were placed in the chambers.

Statistical analysis

Analyses were implemented in R.3.0.0. (R Development Core Team 2013). Experiments one to five were analysed using chi-square tests, with the expectation that with no choice

caterpillars would distribute themselves evenly among choice chambers. Using a power analysis with the expectation that a strong effect size would be required (0.5 for a Chi-square test following Cohen 1988) and setting power at 0.8 and significance at 0.05, I determined that a sample size of 31 would be sufficient (using the pwr package). In all cases I used *c*. 40 caterpillars. Choice six had a 2 × 2 contingency table design and the significance of caterpillar choice compared among material and temperatures using a Fisher's exact test (Sokal and Rohlf 2001).

Results

In experiment 1, caterpillars showed no significant preference for new nest material over empty chambers (Table 1, Fig. 3). Caterpillars likewise showed no preference for new nest material vs. *Sanionia uncinata* moss (experiment 2), or new vs. old nest material (experiment 3) (Table 1, Fig. 3). When acclimated at 0°C for seven days and given a preference between 0°C and 10°C, caterpillars likewise showed no preference. However, when acclimated at 5°C and presented with a choice between 5°C and 15°C, caterpillars preferred the lower temperature (Table 1, Fig. 3). Caterpillars likewise clearly preferred chambers without caterpillars than those with other caterpillars (Table 1, Figure 3). When given a choice of warm vs. cooler old and new nest material, caterpillars again showed no choice (Fisher's Exact Test, $p = 0.105$).

Table 1. Outcome of the chi-squared tests for each of six experiments on *Pringleophaga marioni* caterpillar choice. Under the heading experiment the choices presented to caterpillars are indicated. Significant outcomes are indicated by boldface text.

Experiment	n	Chi-squared test
New nest material Empty chamber	40	$X^2 = 1.6$, d.f. = 1, p = 0.206
New nest material *Sanionia uncinatus* moss	39	$X^2 = 0.2$, d.f. = 1, p = 0.631
New nest material Old nest material	40	$X^2 = 0.4$, d.f. = 1, p = 0.527
Chambers with caterpillars Chambers without caterpillars	40	**$X^2 = 9.1$, d.f. = 1, p = 0.004**
Thermal choices		
0 vs. 10 °C	40	$X^2 = 0.4$, d.f. = 1, p = 0.527
5 vs. 15 °C	30	**$X^2 = 16.1$, d.f. = 1, p < 0.0001**

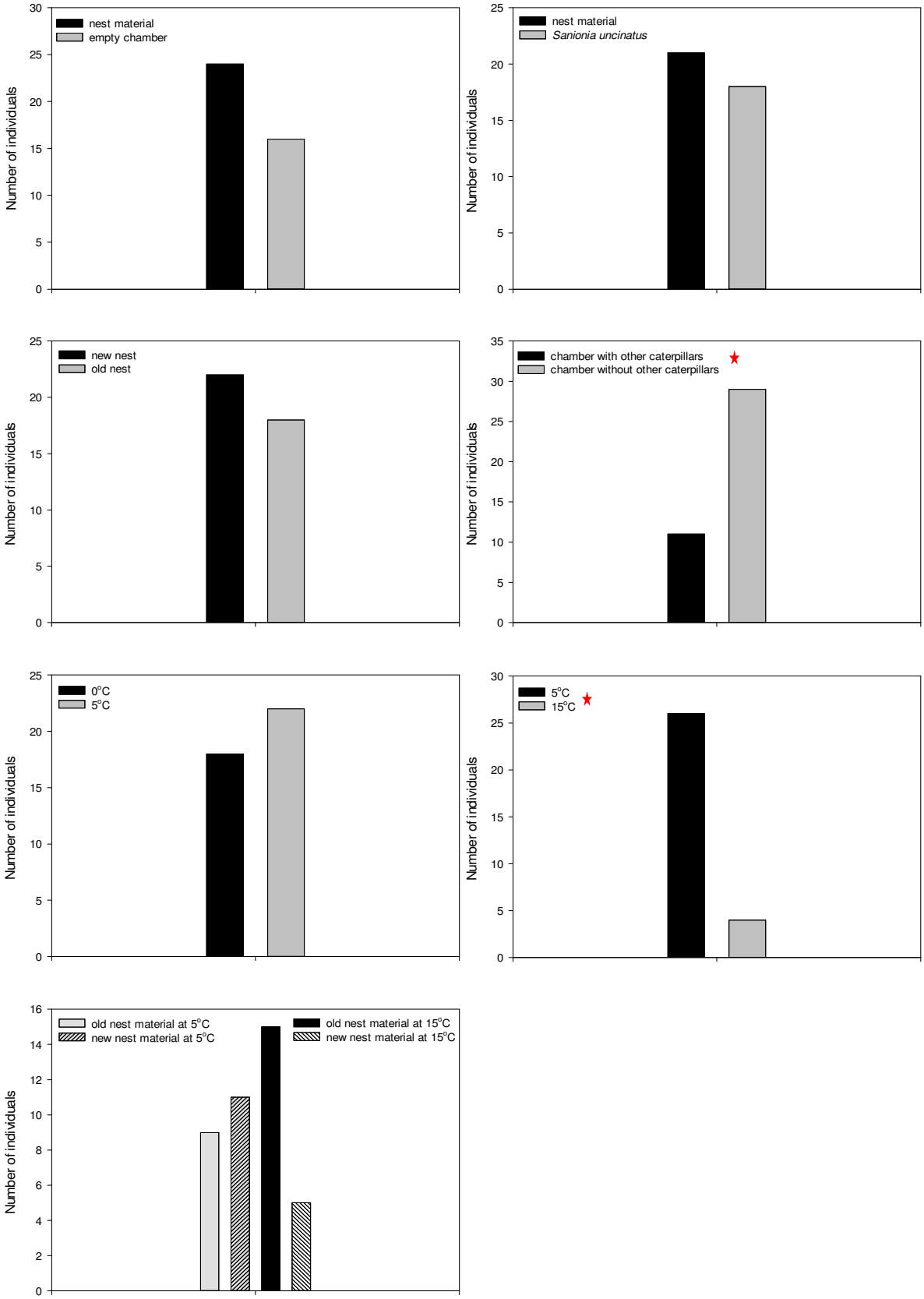


Fig. 3. The number of *Pringleophaga marioni* caterpillars that made a particular choice in each of the experiments conducted to ascertain caterpillar choice. Red stars indicate experiments for which preference was statistically significant (see Table 1 for statistics).

Discussion

The primary aim of this study was to determine whether larvae of *Pringleophaga marioni* actively seek out new wandering albatross nests either on the basis of chemosensory or thermal cues (as may be found in other insects e.g. Bruce *et al.* 2005; Chown & Terblanche 2007; Hamilton *et al.* 2011; Sim *et al.* 2012; Soler *et al*. 2012; Anderson *et al*. 2013). In each of the choice experiments that contrasted new nest material with an alternative (either no material, old nest material or *Sanionia uncinata* moss), there was no significant preference for new nest material. Thus, caterpillars show no active preference for new nest material in the instars examined here.

Although the caterpillars responded under some circumstances to thermal cues, as may take place in other insect species (e.g. Lazzari and Núñez 1989; Flores and Lazzari 1996; Clissold *et al.* 2013), the response was the opposite of that expected. When presented with a choice including a temperature of 15°C, caterpillars showed a significant preference for chambers where temperatures were lower (5°C). If caterpillars encounter significant physiological stress at lower temperatures (Sinclair & Chown 2005) and favour albatross nests for this reason, a preference for higher temperatures is the expectation. Recent work has shown, however, that while optimal movement temperatures are high (*c*. 23°C) (Chapter 5), caterpillars are adversely affected, through low survival, by exposure to 15°C for prolonged periods (Haupt *et al*. 2013). Thus, caterpillars avoid higher temperatures, rather than seeking them out, while here apparently not differentiating between lower temperatures.

Therefore, in these experiments I found no empirical support for active nest-finding ability on the basis of either chemosensory or thermal cues. By contrast, a significant preference was shown by caterpillars for chambers in which no other caterpillars were present. In laboratory studies of *P. marioni*, some captive larvae are omnivorous, including both earthworms and conspecifics in their otherwise typical litter diet (French & Smith 1983; Crafford 1990). Cannibalistic behaviour is not uncommon amongst lepidopteran larvae, particularly under stressful laboratory conditions (reviews in Fox 1975; Pierce 1995; Richardson *et al*. 2010). Indeed, French & Smith (1983) proposed that the cannibalism observed in their "cafeteria" trials of *P. marioni* might have resulted from stress. Active avoidance of conspecifics is consistent with a threat of cannibalism, and may reflect a selective pressure in the field. Given the large numbers of caterpillars in nests such active avoidance may enable co-existence while reducing the risk of injury or death from conspecifics. However, the same might be true of other habitats with high caterpillar densities (see Burger 1978), so precluding any conclusion about a response specifically to conspecific

densities in wandering albatross nests. Nonetheless, large numbers of caterpillars in nests does suggest that some advantage is accruing despite the higher probabilities of conspecific encounters associated with high density.

Overall, it appears that caterpillars do not seek out nest material, but rather tend to avoid deleterious high temperatures and conspecific individuals. Although it is possible that the homogenised nest material used may have lacked specific cues, I deliberately homogenised material from both the inside and outside of nests, and because the nests are constructed from local vegetation, there is no reason to expect any chemical cues to be differentially present in different parts of the nest. Therefore, although caterpillars appear to be capable of responding both to thermal and chemosensory cues, these likely play no role in accounting for high caterpillar biomass and densities in newly abandoned wandering albatross nests. However, nest choice cannot be ruled out entirely for two reasons.

First, first instar larvae may select nests. Choice by first instar larvae cannot be ruled out here because I was unable to undertake tests of them given their scarcity during the period of fieldwork (see Chapter 2: Fig. 2). For a relatively host-specific species the absence of trials in the first instar would be problematic, given the significance of host selection by neonate larvae (Zalucki *et al*. 2002). However, in a polyphagous, broadly-distributed species, where the fitness benefits involve either ongoing nutrient availability or thermal benefits, I expect site selection to continue into the later instars (see e.g. Thompson & Pellmyr 1991; Klok & Chown 1999 for rationale), making the current trials appropriate.

Second, ovipositing female moths might choose nests. However, adult female moths are short-lived (7-11 days) (Haupt *et al.* 2013), sedentary, and thus not easily found in the field (Crafford *et al*. 1986), so also precluding investigation of choice in this stage both here, and likely in the future too (see Chown *et al*. 2002 for data on the decline in abundance of this species). However, the adult choice mechanism could be examined by comparing relatedness of caterpillars in nests to those outside of them using genetic markers. Because females are short-lived and sedentary, and tend to lay their eggs in just a few large batches (Crafford *et al*. 1986; Crafford 1990; Haupt *et al*. 2013), nest selection by females would be expected to result in greater genetic heterogeneity in nests than in similar-sized areas away from them.

Whatever the outcome, the high numbers of caterpillars in nests are unlikely to be as a result of caterpillars actively choosing wandering albatross nests as a preferred habitat. Both the ‘nutrient hypothesis’ and ‘thermal ecosystem engineering’ hypothesis suggest that selection should act on nest-finding ability. Should further investigations reveal similar

findings for adult choice, then these hypotheses are not supported. However, both forms of the incidental hypothesis, i.e. ovipositing moths may distribute their eggs everywhere, or caterpillars or eggs are incorporated into nests during nest building, do not preclude that either thermal benefits or nutrient input may account for the high abundance of caterpillars in nests. Female adult moths are flightless, and often sedentary (Crafford *et al.* 1986), and it is therefore unlikely that they will move too far between the point of emergence and oviposition. Because they lay their eggs in large batches (Crafford *et al.* 1986), incorporation of these eggs into the nest through nest building may likely result in differential larval densities between nests and the surrounding habitats. Thus, these potential mechanisms along with the possibility that nests may serve as a refuge from mice (i.e. 'differential mortality'), would have to be considered more closely.

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Chapter 7: General discussion

"We now know that the ecosystem is not as 'simple' as we once thought…it is up to us to discover, quantify and marvel at those interactions. The island remains a superb laboratory for ecological research"

(Smith 2008 on Marion Island)

Nesting wandering albatross, *Diomedea exulans*.

7.1. Thesis summary

When a pelagic predator not only contributes nutrients to a system (Burger *et al.* 1978; Smith 1978), but also presumably acts as an ecosystem engineer for a species identified as keystone in ecosystem functioning (Smith & Steenkamp 1992; 1993), a more complex interaction could not be envisaged. Flightless moth caterpillars, *Pringleophaga marioni*, have much higher and considerably less variable populations in recently abandoned wandering albatross (*Diomedea exulans*) nests compared to most habitats on Marion Island. Since no evidence for nutrient input was provided (see Joly *et al.* 1987), a role for temperature was suggested given that occupied nests are substantially warmer than surrounding habitats (Sinclair & Chown 2006). The primary goal of this thesis was to better understand the reason for the high abundance of caterpillars in nests, and explore the role of the wandering albatross as thermal ecosystem engineers to *P. marioni* caterpillars. Possible explanations that may account for the high numbers of caterpillars in nests were identified, as well as hypotheses, which if met, would provide support for the thermal ecosystem engineering hypothesis proposed by Sinclair & Chown (2006) (Fig. 1). These were tested by adopting a multidisciplinary approach in which physiology, ecology and behaviour were integrated:

Resolving the life-cycle paradox of Pringleophaga marioni

In C**hapter 2**, caterpillars were reared at varying temperature regimes and a more quantitative life cycle estimate for *P. marioni* was made. This was one year, contrary to previous estimates of between two to five years (Crafford *et al*. 1986). Measurements of head capsules from a field survey in 1983-1984 (Crafford 1990) also suggest that cohorts with a one year life cycle are present. Larval development time was similar at the fluctuating temperatures of 5-15°C and 10°C (i.e. 37 and 46 weeks respectively), with a longer duration at 5°C (66 weeks). While development was fastest at 15°C (16 weeks), caterpillars were adversely affected at high temperatures.

Caterpillar abundance and temperature in nests

In **Chapter 3**, caterpillar abundance was examined in three nest types (old, fledged, new), and temperatures within nests and surrounding habitats were examined for most of the duration of nest occupancy. Caterpillar abundance was significantly higher in new nests compared to old and fledged nests. Moreover, the low variation in caterpillar biomass in new nests compared to other vegetation complexes suggested that caterpillars are distributed more evenly within new nests than any other habitat on Marion Island. The year-long temperature

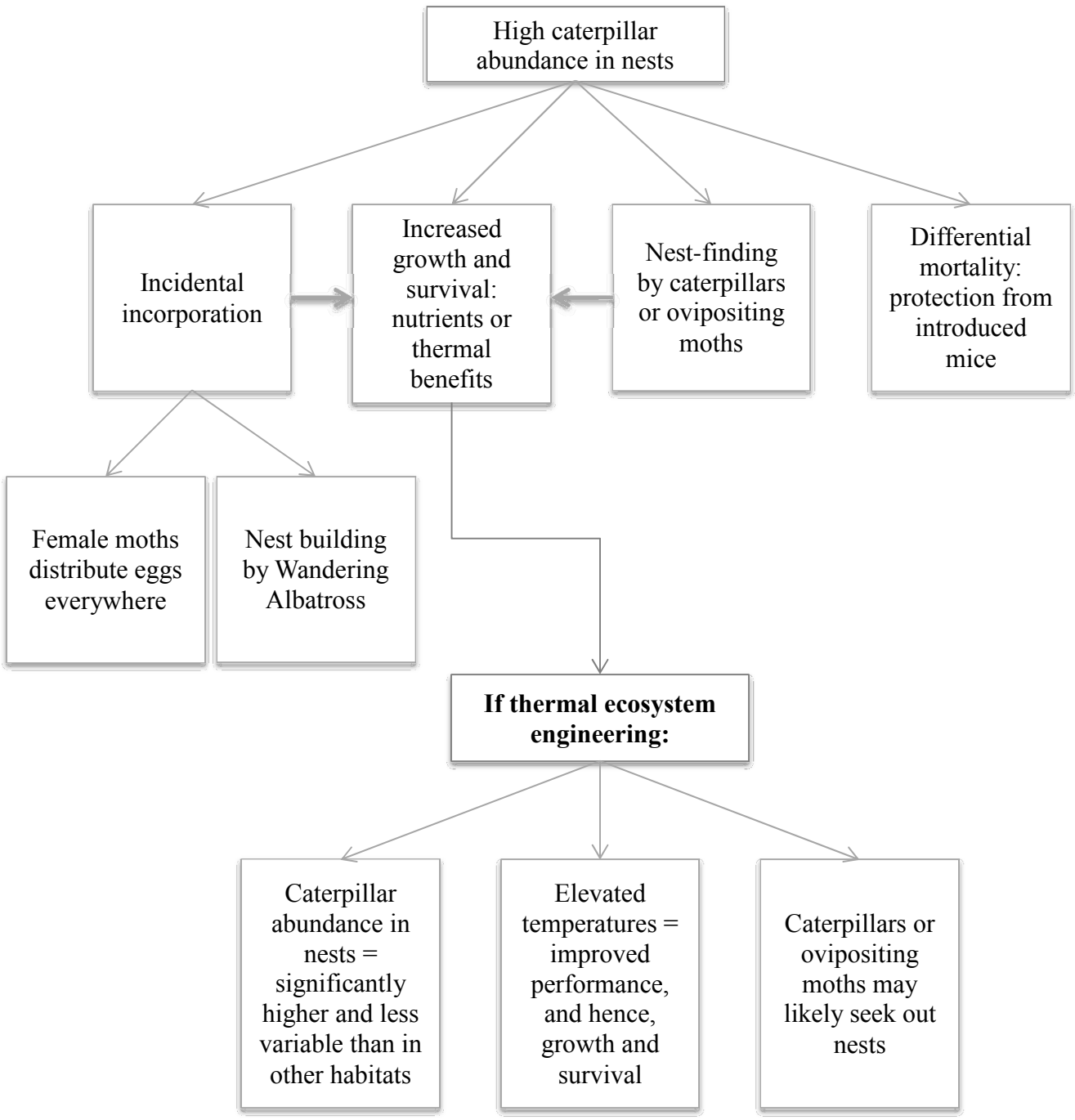

Fig. 1. Explanations that may account for the high caterpillar abundance in nests and hypotheses tested to examine the hypothesis of thermal ecosystem engineering.

data revealed that temperatures were significantly higher during nest occupancy compared to other habitats. In particular, daily minimum and absolute minimum temperatures were significantly higher in occupied nests compared to all other habitats and temperature events at which *P. marioni* experience chill coma were substantially reduced.

The physiological and behavioural responses of Pringleophaga marioni

In **Chapter 4**, I examined the effects of thermal acclimation on the metabolic response of *P. marioni* caterpillars. Whether water loss influenced the metabolic response, as well as the effects of fluctuating temperatures, were also examined. Partial support was found for metabolic cold adaptation (Addo-Bediako *et al.* 2002; Clarke 2003), with little evidence that caterpillars depress their metabolism to conserve water (Chown 2002). In addition, fluctuating acclimation temperatures (5-15°C) resulted in metabolic depression, and this was most likely as a consequence of metabolic costs at high temperature extremes.

In **Chapter 5**, the relationship between preference and locomotor performance of *P. marioni* was examined. Compared to the optimal movement temperature (*c.* 23°C), preference was far lower at 4.6°C to 8°C. These temperatures more closely resembled temperatures at which survival was high (Haupt *et al.* 2013), or where feeding is optimal (Crafford 1990), suggesting that thermal preference may differ depending on the physiological trait examined. Thermal acclimation had no significant effect on locomotor performance traits (i.e. the optimum temperature, maximum speed and the performance breadth), nor on thermal preference. This indicates a lack of phenotypic plasticity for *P. marioni*, a feature characteristic of many invertebrates on Marion Island (e.g. Deere *et al.* 2006; Slabber *et al.* 2007; Marais *et al.* 2009), and largely as a result of unpredictable climatic events to which acclimation attempts would be futile (Deere & Chown 2006). Exposure to the fluctuating temperature of 5-15°C resulted in a significantly lower performance breadth compared to 10°C (closest comparable mean temperature). In addition, caterpillars acclimated at 5-15°C preferred lower temperatures of 4.6°C, suggesting that these organisms may compensate for exposure to unfavourable environments by seeking out lower temperatures (Gvoždik *et al.* 2007; Qu *et al.* 2011).

Choice or chance?

Favourable conditions proposed for caterpillars within wandering albatross nests (Joly *et al.* 1987; Sinclair & Chown 2006), suggest that ovipositing female moths or caterpillars may likely seek out nests. Alternatively, incidental incorporation of caterpillars or eggs into nests

(i.e. via nest building, or the random distribution of eggs by ovipositing females) (Fig. 1), does not preclude further benefits, and may also be the initial mechanism by which the high caterpillar biomass in nests are realized. The hypothesis that caterpillars may use chemosensory or thermal cues to locate nests, as may be found in other insects (e.g. Bruce *et al.* 2005; Chown and Terblanche 2007; Hamilton *et al.* 2011; Sim *et al.* 2012; Soler *et al.* 2012; Anderson *et al*. 2013), was tested in **Chapter 6**. No preference for new nest material was found, and caterpillars showed a significant avoidance of high temperatures and conspecifics. Consequently, empirical evidence suggests that caterpillars likely do not seek out albatross nests.

7.2 How do caterpillars populate nests?

Propositions of nutrient (Joly *et al.* 1987) and thermal benefits (Sinclair & Chown 2006) were used to explain the high occurrence of *Pringleophaga* caterpillars in wandering albatross nests, however, the exact mechanism by which caterpillars arrive in nests is not known. Although caterpillars appear unlikely to seek out nests (Chapter 6), whether ovipositing female moths may preferentially search for nests may also account for the high caterpillar abundance in nests compared to other vegetation communities. Given sufficient time spent searching for adult female moths, choice experiments could also show whether nest-finding by moths may occur. Alternatively, whether female moths preferentially oviposit eggs on nests may also be tested through genetics. Because females lay their eggs in just a few large batches (Crafford *et al*. 1986; Crafford 1990; Haupt *et al*. 2013), nest selection by females would be expected to result in greater genetic heterogeneity in nests than in similar-sized areas outside nests. In the former situation, several different females would be expected to oviposit in a given nest following attraction to it. In the latter, it might be expected that a more limited number of females would lay in a given area meaning substantial spatial structure in genetic variation. Likewise, it seems plausible that if albatrosses are selecting vegetation with caterpillars contained within it, relatedness might be lower than if females were actively attracted to nests. However, this would depend also on the circumstances of nest building which have not been examined from such a perspective (Warham 1997).

Incidental incorporation via nest-building may also account for the high caterpillar abundance in nests compared to non-nest habitats. Although caterpillar biomass was not consistently higher in nests compared to other habitats, variance of caterpillar biomass was lower in new nests than in any other habitat (Sinclair & Chown 2006; Chapter 3). This suggests that there is a greater chance of finding caterpillars in new nests than anywhere else

on Marion Island. Moreover, caterpillar abundance was significantly higher in new nests compared to old and fledged nests. The recently abandoned nests sampled in this study, and by Sinclair & Chown (2006), were nests built for that particular breeding season in mid-summer of the year prior to sampling (Ryan & Bester 2008). Wandering albatrosses are essentially accumulators, and grab the top few centimetres of material from a wide area to build their nests. Since invertebrates are usually found in the first few centimetres of soil, it is highly probable that *P. marioni* caterpillars or eggs may be incorporated into nests during nest building. Nests are also built in early summer (Ryan & Bester 2008) when first instar larvae of *P. marioni* reach their peak densities on Marion Island (Chapter 2) and this may also contribute to the high numbers of caterpillars found in new nests.

If *P. marioni* caterpillars or eggs are incorporated into nests during nest-building, then it is predicted that caterpillar abundance in nests and areas adjacent to nests should be similar if not equal. By correcting appropriately for area sampled and the volume of the nest, this hypothesis may be tested by a direct comparison of caterpillar abundance in nests and areas adjacent to nests. However, egg survival may also be higher in nests compared to non-nest habitats, and because eggs may be hard to find using the above method, another alternative would be to manually build nests by picking up clumps of vegetation, piling these together into a nest-like structure, and then counting the numbers of caterpillars after some time. If a substantial number of caterpillars or eggs are incorporated into nests in this way, then it is likely that the nesting activities of wandering albatross may be the initial mechanism by which caterpillars arrive in nests.

Neither nest-finding by ovipositing female moths, incidental incorporation by ovipositing females, nor incidental incorporation via nest-building preclude further benefits by either thermal ecosystem engineering or nutrient availability (Fig. 1). That caterpillars are willing to risk encounters with conspecifics to which they showed a strong avoidance to (Chapter 6), implies that some advantage must be accruing. Similarly, if ovipositing female moths are likely to seek out nests, the assumption is that females will choose habitats that will maximise offspring performance (Thompson 1988; Nylin & Janz 1993; Huk & Kühne 1999; Scheirs *et al*. 2000; Ganehiarachchi *et al*. 2013).

7.3. Are wandering albatross thermal ecosystem engineers?

If wandering albatross are thermal ecosystem engineers, I predicted that caterpillar abundance would be higher in nests whilst variance in biomass would be low compared to other habitats (Fig. 1). Caterpillar abundance was not always consistently higher in nests compared to other

habitats, although variance in caterpillar biomass was far lower, suggesting that caterpillars are indeed distributed more evenly in nests compared to any other habitat on Marion Island (Chapter 3; Sinclair & Chown 2006). I also predicted that ovipositing female moths or caterpillars may likely seek out nests (Fig. 1). Caterpillars are unlikely to use thermal or chemosensory cues to seek out nests, but this does not preclude further benefits after incidental incorporation of caterpillars into nests, or the chance that ovipositing female moths may seek out nests as a suitable habitat with fitness benefits for their offspring (Chapter 6).

Lastly, I predicted that elevated temperatures would result in differential performance between nests and non-nest habitats (Fig. 1). In some instances, high temperatures had significant deleterious effects on caterpillar performance. For example, although development was optimal at high temperatures of 15°C, caterpillars were adversely affected by low survival (Chapter 2). Moreover, caterpillars showed a significant preference for lower temperatures (Chapter 5) and a strong avoidance of high temperatures (Chapter 6). These findings are therefore contrary to the expectation given that caterpillars may favour albatross nests because they encounter significant physiological stress at lower temperatures (Sinclair & Chown 2005). However, although high temperatures may occasionally be reached in wandering albatross nests, temperatures are not consistently high, and there is considerable daily fluctuation (Chapter 3; Sinclair & Chown 2006).

Fluctuating temperatures are a predominant feature of the natural environment and studies have shown that the warm periods of fluctuating thermal regimes may provide a respite for insects, enabling them to repair cellular-level damage after exposure to cold conditions (e.g. Renault *et al.* 2004; Lalouette *et al.* 2011), but there are also indications of negative effects of warm exposure (e.g. Williams *et al.* 2012). Fluctuating temperatures of 5-15°C did result in significant metabolic depression (Chapter 4), and restricted the range over which performance may be achieved (i.e. significantly lower performance breadth at 5-15°C; Chapter 5). Variable temperature conditions simulated in the laboratory (e.g. 5-15°C) provide a representative model of possible temperature interactions in the nests (Chapter 3; Sinclair & Chown 2006), but there are differences. In laboratory acclimations, caterpillars spent a longer time at each temperature extreme compared to what would be expected in the nests. In the nests, the caterpillars may experience greater daily temperature fluctuations with less time spent at extreme temperatures. For example, the range of daily minimum and maximum temperatures in nests are relatively large, and the difference between daily minimum and maximum temperatures may range anywhere between 2°C and 8°C depending on the time of the year (see Table 5 in Chapter 3). Time-temperature interactions may have significant

effects on caterpillar performance, and variable conditions in nests may not have the same deleterious effects, with warm periods of shorter duration possibly providing opportunities for improved performance.

The variable temperatures including high temperatures of 15°C did not have deleterious effects on caterpillar growth rates and survival, and larval development occurred faster at a fluctuating temperature of 5-15°C compared to a lower temperature of 5°C (Chapter 2). Furthermore, although acclimation to high temperatures (e.g. 15°C) did not result in maximal performance, a high optimal movement temperature of *c.* 23°C suggests that caterpillars may be more active in warm nest temperatures (Chapter 5). Moreover, even though *P. marioni* caterpillars are freeze tolerant, they do experience low temperature-related reductions in growth rate (Klok & Chown 1997; Sinclair & Chown 2005; Haupt *et al.* 2013) and the year-long temperature data has shown that daily minimum and absolute minimum temperatures are significantly higher in occupied nests compared to other habitats on the island, and chill coma events also occur less often in these warm microhabitats (Chapter 2). These findings suggest that the thermal engineering of wandering albatrosses may very well improve caterpillar growth and survival and may therefore contribute to the high abundance of caterpillars found in nests.

The effects of thermal ecosystem engineering may however also manifest in many different forms. For example, decomposition processes in the Antarctic are slow as a result of low temperatures, and warming tends to increase nutrient availability and enhance decomposition rates (Schmidt *et al.* 2002; Hill & Henry 2011; Nielsen & Wall 2013). A recent study showed that soil microbial communities in sub-Antarctica and Antarctica responded to increasing temperatures with an increase in both bacterial and fungal abundances, and greater nutrient availability was likely linked to changes in bacterial community composition (Yergeau *et al.* 2012). This is not surprising given that Antarctic soil microorganisms have optimal growing temperatures far above current field conditions (Rinnan *et al.* 2009), thus enabling them to benefit rapidly from elevated temperatures (Wynn-Williams 1996; Yergeau *et al.* 2012). An increase in microbial biomass may likely be able to support more soil invertebrates (review in Convey & Smith 2006; Nielsen & Wall 2013). Thus, under elevated temperature conditions found in occupied wandering albatross nests, microbial communities may flourish, and in turn, nest material may become a highly favoured food-source, driving caterpillar survival, and hence, the high abundance of caterpillars in nests.

Testing the potential for thermal ecosystem engineering in the field

Field manipulative experiments in which temperatures are increased have been used to investigate the effects of warming on a range of organisms from insects (Wu *et al.* 2012) and marine organisms (Martínez *et al.* 2012; Galaiduk *et al.* 2013) to plants (Fang *et al.* 2013; Xu *et al.* 2013; Zheng *et al.* 2013). Likewise, in response to profound warming patterns in the Antarctic, experimental manipulations of soil and plant associated microarthropod communities have also been conducted (review in Convey & Smith 2006; e.g. Kennedy 1994; Convey *et al.* 2002; McGeoch *et al.* 2006; Slabber *et al.* 2007; Bokhorst *et al.* 2009; Day *et al.* 2009; Bokhorst *et al.* 2011; 2013; Dennis *et al.* 2013). Thus, perhaps a more direct approach to testing whether elevated temperatures result in significantly higher numbers of caterpillars in nests would be to build and artificially incubate nests on Marion Island.

If elevated temperatures conferred some advantage for caterpillars, then caterpillar biomass in incubated nests would be higher than in control nests (i.e. no heat source). In this way, the effects of increasing temperatures on the composition and abundance of microbial communities could also be determined. Sampling nests that are occupied by birds is another option, but this is unlikely to occur without destroying the nest structures and disturbing the birds. Alternatively, researchers that overwinter on Marion Island could sample occupied nests immediately after chicks fledge in December or January. The 'fledged' nests in this study were sampled in April and May during the annual relief voyage. These nests were vacated at least three months prior to sampling and were therefore not good proxies for occupied nests.

Do mice play a role in the high numbers of caterpillars in nests?

On Marion Island, introduced house mice, *Mus musculus*, take various weevil species and *P. marioni* caterpillars as their preferred prey items (Rowe-Rowe *et al.* 1989; Chown & Smith 1993; Smith *et al.* 2002). Another hypothesis that may explain the high occurrence of caterpillars in nests is that differential mortality in and out of nests occurs as caterpillars may be consumed less often by mice while in nests.

Many studies have used animal exclosures to exclude predators or introduced species to determine, either, their effects on local fauna or flora (e.g. Anderson & Folk 1993; Bricker *et al.* 2010; Lettink *et al.* 2010), or, their relative importance to a particular system (e.g. Westerman *et al.* 2003). Often, the exclusion of target species has resulted in increases in local species assemblages and composition (Keesing 1998; Parsons *et al.* 2013). On sub-antarctic Macquarie Island, exclosures were used to show that introduced ship rats, *Rattus*

rattus, have negative effects on seedling recruitment and distribution of an important megaherb, *Pleurophyllum hookeri* (Shaw *et al.* 2005). On Marion Island, excluding mice had no effect on either invertebrate abundance, or vegetation communities, but this may have been as a result of low statistical power (i.e. five exclosures and five control plots) or mice occasionally entering exclosures (Van Aarde *et al.* 2004).

By constructing robust mouse exclosures around recently abandoned wandering albatross nests, the hypothesis that differential mortality of caterpillars occur in and out of nests as a result of mouse predation, can be tested. If nests are serving as refuges against mouse predation, then the expectation is that caterpillar abundance will be highest in nests where mice have been excluded compared to nests that are open to predation. In addition, on mouse-free Prince Edward Island, populations of *Pringleophaga* in vegetation complexes are substantially higher compared to on Marion Island (Crafford & Scholtz 1987). Thus, if caterpillars occur more abundantly in vegetation complexes compared to in wandering albatross nests on Prince Edward Island, then evidence may be provided that the high occurrence of caterpillars in nests on Marion Island is as a result of decreased predation by mice.

7.4. The broader significance of the work

Studies of exemplar taxa such as *Pringleophaga* that are abundant on sub-Antarctic islands (Crafford *et al.* 1986) and which are crucial for ecosystem functioning (Smith & Steenkamp 1992; 1993), are useful for estimating the effects of perturbations. First, a life cycle estimate for *P. marioni* of one year, contrary to previous estimates of between two to five years (Crafford *et al*. 1986), suggests that population replacement is faster than has previously been assumed. This may explain why this species has not yet gone extinct despite extremely high estimates of predation by house mice (Chown *et al.* 2002; Smith *et al.* 2002). This does not mean that *P. marioni* is not at risk from predation by mice, since caterpillars remain the preferred prey species (Smith *et al.* 2002) and census estimates indicate a declining population (Chown *et al.* 2002). However, formal models may now use this more quantitative life cycle estimate to obtain a more accurate population estimate for this species, as well as to model the outcome of conservation programmes to eradicate mice. Thus, given that low population estimates are found, the potential now exists for a more formal assessment of this species according to the IUCN red list criteria. No such assessment has been made for any plant or invertebrate species in the sub-Antarctic.

The polar regions are also experiencing rapid climate change with implications for both terrestrial and marine systems (reviews in Convey & Smith 2006; Convey *et al.* 2009; Nielsen & Wall 2013). Evidence exists that climate change occurs in the polar regions at faster rates than elsewhere (Turner *et al.* 2009). Since the 1950s, the Antarctic region has seen a significant increase in atmospheric temperature (Turner *et al.* 2005; Steig *et al.* 2009) with an average increase of 0.2°C since the late 19th Century (Convey *et al.* 2009). Average sea temperatures in the Southern Ocean have also increased (Böning *et al.* 2008), and a global rise in sea levels of 2.5 mm yr^{-1} has been recorded (Jacobs 2006; Cazenave *et al.* 2009). These climatic changes have had negative effects on terrestrial and marine communities (Forcada *et al.* 2005; 2006; Clarke *et al.* 2007; Barnes & Peck 2008; Montes-Hugo *et al.* 2009), as well as indirect effects as warming temperatures may facilitate the introduction of alien species (Bergstrom & Chown 1999; Frenot *et al.* 2005; Chown & Convey 2007; Treasure 2012).

The growth, life cycle patterns, survival and reproduction of many plants and animals are therefore dependent on regional temperature regimes (Chown & Nicolson 2004), making the physiological and behavioural response of organisms to changing temperatures a primary concern. Ultimately, organisms that have a low tolerance for warming, limited acclimation ability, and reduced dispersal, are at greatest risk of species extinction from rapid climate change (Deutsch *et al*. 2008). A large mismatch between the thermal optimum and preferred temperature of *P. marioni* is in keeping with high latitude temperate species (Deutsch *et al.* 2008). High optimum temperatures compared to body temperatures suggests that high latitude species will be better able to cope with rising temperatures, compared to more tropical species that are already living close to their critical maximum temperatures (Deutsch *et al.* 2008). Here, I've shown that although the optimum temperature for locomotion of *P. marioni* was far higher than preferred temperatures, thermal preference may be more matched to the temperatures at which survival or feeding is maximised (Crafford 1990; Haupt *et al.* 2013), suggesting that optimum temperatures may differ for different physiological traits (Huey & Stevenson 1979), and this should be taken into account when predicting climate change scenarios for high latitude species. The lack of phenotypic plasticity found for locomotor performance in *P. marioni*, and the finding that their survival is compromised at high temperatures of 15°C, may already suggest that this species may not be well equipped to cope with increasing temperatures.

Furthermore, thermoregulatory behaviour has also been demonstrated to have a direct link to nutritional state (Coggan *et al*. 2011). At low temperatures, some insects experience

slower growth rates than they would at higher temperatures, but nutrient utilisation and efficiency increases (Miller *et al*. 2009; Coggan et al. 2011). Whether the general preference for low temperatures in *P. marioni* and other species (e.g. Martin & Huey 2008), may be linked to assimilation efficiency, should also be considered. Thus, studies investigating the relationship between thermoregulatory behaviour and thermal physiology should take into account that preference may differ depending on the ecological importance of varying physiological traits.

Evidence for partial metabolic cold adaptation suggests that caterpillars of *P. marioni* are part of an extensive group of species showing such adaptive strategies to cold environments (Block & Young 1978; Aunaas *et al.* 1983; Strømme *et al.* 1986; Chown *et al.* 1997). Furthermore, since biotic impacts of warming are mediated through physiology, and metabolic rate is a fundamental measure of physiological activity and ecological impact (Brown *et al.* 2004), the effects of increasing temperature on organisms will likely manifest in their metabolic responses (Dillon *et al.* 2010). High latitude regions experience a greater shift in warming compared to more tropical areas (IPCC 2007). Metabolic rate increases exponentially with temperature, and recent work has suggested that because of the nature of the metabolic rate temperature relationship, the effects of increasing temperatures should be more pronounced in tropical organisms that are already living at warm baseline temperatures, compared to organisms in cooler regions (Dillon *et al.* 2010). However, climate change scenarios do not only predict a general warming trend, but also an increased variability in weather conditions (IPCC, 2007). The significant metabolic depression at fluctuating temperatures observed in this study, suggests that cold environment species may be more sensitive to rising temperatures without taking environmental variability into account.

On Marion Island, any direct impact of climate change on the *P. marioni* population may have serious indirect implications for ecosystem functioning. For example, increasing temperatures (Smith & Steenkamp 1990; Melice *et al.* 2003; Le Roux & McGeoch 2008) would also mean an increase in primary productivity, and thus, a greater resource demand. This sequence of events could change nutrient cycling, and because of *P. marioni's* keystone role (Smith & Steenkamp 1992; 1993), functioning of the system.

7.5. Concluding remarks

This study has contributed significantly to the examination of the role of wandering albatross as thermal ecosystem engineers to *P. marioni* caterpillars, as well as the mechanisms by which this engineering may take place. Firstly, while caterpillars themselves are unlikely to

seek out nests, I have identified several other mechanisms by which caterpillars may arrive in nests and have suggested methods by which these ideas may be tested. Secondly, while caterpillars in nests may accrue direct benefits from the warm thermal environment, I have also highlighted how the effects of thermal ecosystem engineering may manifest in different forms. Consequently, factors other than thermal benefits that may also explain the high abundance of caterpillars in nests were identified. It is therefore apparent that much scope for future work exists to better understand the complex interaction that exists between wandering albatross and flightless moth caterpillars, and this thesis has highlighted the significance of integrating disciplines to do so. Moreover, this work has contributed significantly to understanding how *P. marioni* caterpillars may respond both physiologically and behaviourally to changing temperature conditions. This is of particular importance in a region that is facing rapid climate change. The high abundance of caterpillars in nests suggests that these microhabitats are indeed a crucial source for the *P. marioni* population, and perhaps the focus should now be on how significant the contribution of caterpillars within nests is to the flightless moth population as a whole. Given ongoing climate change scenarios and predation of *P. marioni* by mice, this is a non-trivial question of potential relevance to long term ecosystem management on Marion Island.

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